Effects of Cueing Foreign Vocabulary on Memory Processes during Wakefulness and Sleep

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Abstract

Sleep plays a crucial role for memory consolidation. It is assumed that the memory function of sleep is based on spontaneously occurring hippocampal memory reactivations during non rapid-eye movement sleep (Non-REM). A causal role of such memory reactivations is supported by studies showing that experimentally inducing reactivations during Non-REM sleep by using associated memory cues benefits memory consolidation. The present thesis describes a number of studies closely investigating the effects of reactivating foreign vocabulary during sleep and wakefulness.

Study I investigated whether verbal cueing during Non-REM sleep would improve vocabulary learning. Replay of Dutch words during sleep improved later memory for the German translations, while no memory benefit emerged during active and passive waking. On a neural basis, successful cueing during sleep was associated with a pronounced frontal negativity in event related potentials, a higher frequency of frontal slow waves as well as cuing-related increases in oscillatory theta power.

Study 2 explored whether replay of congruent and incongruent Dutch German word pairs during sleep would lead to enhanced or deteriorated recall performance, respectively. While cueing of single words enhanced memory performance, cue + feedback replay did not exert any behavioural effect, independent of content. Successful single word cueing was associated with increased oscillatory theta and spindle activity as well as a higher frequency of frontal slow waves. Neural patterns in the theta and spindle range vanished when feedback cues were presented, indicating that the presentation of a second stimulus might have blocked reactivation-associated processes, thereby leading to the lack of any behavioural effect.

Study 3 investigated in depth the sleep specificity of vocabulary cueing by excluding tiredness as confounding factor in wake control participants. Thus, the effect of vocabulary cueing during active and passive daytime wakefulness was assessed. Again replay of Dutch words did not improve later memory for the German translation in both of the waking groups, indicating that the beneficial effects of cueing seem to be solely sleep specific.

Study 4 was designed to investigate whether cueing during sleep would lead to enduring changes in oscillatory theta power at the time of subsequent recognition testing. Replayed words elicited stronger parietal theta power during recognition as compared to non-cued words. This result suggests that the reactivation-induced increase in theta activity might reflect a strengthening of individual memory traces by cueing during sleep.

Summarizing, the present thesis investigated the cueing of foreign vocabulary during wakefulness and sleep and associated neural activity. The obtained results demonstrate the possibilities and potential practical relevance of cueing foreign vocabulary during sleep. Still, many important topics are unknown and wait for clarification.

Zusammenfassung

Schlaf spielt eine wichtige Rolle bei der Konsolidierung von Gedächtnisinhalten. Es wird angenommen, dass die Gedächtnisfunktion des Schlafes auf spontanen hippokampalen Reaktivierungen zuvor gelernter Inhalte beruht. Eine kausale Rolle dieser Gedächtnisreaktivierungen konnte durch verschiedene Studien bewiesen werden, die zeigen konnten dass Reaktivierungen im Schlaf durch das wiederholte Darbieten assoziierter Reize induziert und dadurch die Gedächtnisleistung verbessert werden kann. Die vorliegende Arbeit beschreibt eine Reihe von Studien die sich mit den Effekten der Reaktivierung von fremdsprachigen Vokabeln im Schlaf und Wachzustand beschäftigen.

Die erste Studie untersuchte, ob das Einspielen von Vokabeln im Non-REM Schlaf das Lernen von Vokabeln verstärkt. Im Vergleich zu nicht reaktivierten Worten verbesserte das wiederholte Einspielen holländischer Vokabeln im Schlaf die Erinnerung an die deutschen Übersetzungen. In einer aktiven und passiven Wachgruppe zeigten sich im Gegensatz dazu keine gedächtnisförderlichen Effekte. Auf neuronaler Ebene war das erfolgreiche Einspielen von Vokabeln mit einer verstärkten frontalen Negativität in den ereigniskorrelierten Potentialen, einer größeren Anzahl an langsamen Oszillationen und einer verstärkten Theta-Aktivität verbunden.

Die zweite Studie untersuchte ob das Einspielen kongruenter und inkongruenter holländisch-deutscher Wortpaare zu einer verbesserten bzw. verschlechterten Gedächtnisleistung führen würde. Während die Reaktivierung einzelner holländischer Wort wieder mit einer verbesserten Erinnerungsleistung einherging, zeigte das Einspielen von Wortpaaren, unabhängig vom Inhalt keinen Einfluss. Erfolgreiche Einzelwort-Reaktivierungen waren verbunden mit verstärkter oszillatorischer Aktivität im Theta und

Spindelbereich, sowie einer größeren Anzahl an langsamen Oszillationen. Wurde jedoch ein zweites Wort eingespielt verschwand dieses Muster an oszillatorischer Aktivität im Theta und Spindelbereich. Dieses Ergebnis weist darauf hin, dass die Präsentation eines zweiten Stimulus reaktivierungsbezogene Prozesse blockiert haben könnte, was sich auch in den fehlenden Verhaltenseffekten widerspiegelt.

Studie 3 untersuchte im Detail die Schlafspezifität von Vokabel-Reaktivierungen. Dabei wurde das Einspielen von Vokabeln an wachen Versuchspersonen untertags durchgeführt um Müdigkeit als konfundierenden Faktor ausschließen zu können. Jedoch auch an gut ausgeruhten VersuchsteilnehmerInnen zeigte sich keine gedächtnisförderliche Wirkung des Vokabeln-Einspielens. Damit kann geschlussfolgert werden, dass die förderliche Wirkung der Vokabelreaktivierung rein schlafspezifisch ist.

Schlussendlich wurde Studie 4 konzipiert um zu untersuchen ob das Einspielen von Vokabeln im Schlaf auch Einfluss auf Theta Aktivität in Bezug auf eine nachfolgende Wiedererkennungsaufgabe hat. Im Schlaf eingespielte Wort lösten im Vergleich zu nicht eingespielten Worten, verstärkte Theta Aktivität beim nachfolgenden Wiedererkennen aus. Dieses Ergebnis dürfte auf eine Stärkung der individuellen Gedächtnisspur durch die Reaktivierung hinweisen.

Die vorliegende Dissertation untersuchte die Einflüsse des Cueings von fremdsprachigen Vokabeln im Schlaf und Wachzustand, sowie assoziierte neuronale Aktivitäten. Die Resultate weisen auf die potentiellen Möglichkeiten und die praktische Relevanz des Einspielens von Vokabeln im Schlaf hin, während viele Fragen noch ungeklärt sind.

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ONE

Introduction

1.1 Background

The ability to form and recall memories is a quintessential capability of any being in order to cope with an ever-changing environment. Without memory we would not be able to remember our past, master the present or anticipate the future. Thus, memory is not only fundamental to our everyday lives, it defines who we are. Sleep seems to play a crucial role in memory formation. While sleep has been long perceived as a passive state, given the accompanying relative inactivity, the loss of consciousness and the reduced responsiveness to external events, growing evidence suggests that the sleeping brain is anything but passive. The following sections will provide an overview concerning several cognitive processes acting during sleep. The primary focus will thereby lie on the memory function of sleep and how and to what extend such processes can be externally influenced. In this context the sleeping brains capacity to perceive and process external stimuli as well as its role in language learning will be evaluated.

1.2 Sleep and Memory

Memory evolution can be divided into three core processes, namely encoding, consolidation and retrieval (Gabrieli, 1998). During encoding, new and initially labile memory traces are formed. Hence at an early stage memories are still fragile and susceptible to decompose. Consolidation refers to a process, which is thought to strengthen and stabilise those newly encoded memories. Thereby new memories are integrated into preexisting knowledge networks, making them more resistant against decay. Retrieval characterises processes of accessing and recalling stored memories.

While encoding and recall are clearly tied to the state of wakefulness, sleep has been repeatedly proven to play a crucial role in the consolidation of prior learned memories

(Diekelmann & Born, 2010; Rasch & Born, 2013). Beneficial effects of sleep on memory have been demonstrated in various species, among others insects, rodents and humans (Vorster & Born, 2014) and for diverse types of memories (Walker & Stickgold, 2004). In humans such strengthening effects of sleep have been shown for instance with regards to declarative (Gais & Born, 2004) and procedural memories (Werner Plihal & Born, 1995), priming (W Plihal & Born, 1999) and even conditioning (Menz et al., 2013). Furthermore, sleep has been demonstrated to support processes of abstraction, inference, and insight (Ellenbogen, Hu, Payne, Titone, & Walker, 2007; Lewis & Durrant, 2011; Wagner, Gais, Haider, Verleger, & Born, 2004). Hence the question arises how sleep might accomplish such beneficial effects on memory processes, especially related to consolidation.

First concepts almost a century ago assumed that sleep might convey its memory function rather passively (Jenkins & Dallenbach, 1924), since it was assumed to represent a state where no encoding of external information takes place. Thus, sleep was suggested to protect memories from interference like a passive shelter. The beneficial effects of sleep on memory performance were assumed to last only until exposure to new interference in the next day (Ellenbogen, Payne, & Stickgold, 2006). However, increasing evidence has accumulated that the memory function of sleep goes far beyond these early assumptions (e.g. Gais et al., 2006; Born and Wilhelm, 2012; Walker and Stickgold, 2004), implicating that sleep might play a rather active role when it comes to memory processing. The next section will describe in detail the active system consolidation theory, which accounts for this active role of sleep in memory formation, by integrating a vast variety of experimental findings in humans and animals.

1.3 Reactivation of memories and the active system consolidation theory

The active system consolidation theory, conceptually based upon the assumptions of the standard two-stage model of memory consolidation (Marr, 1971; McClelland, McNaughton, & O'Reilly, 1995) assumes the existence of two distinct memory stores. One is supposed to be fast learning and thereby acting as a temporary store, while the other learns at a slower rate and serves as a long-term store. It is suggested that new memories are initially encoded in parallel into both the fast (the hippocampus when it comes to declarative memories) as well as the slow learning store (i.e., the neocortex). While the hippocampus warrants quick encoding of new memories, these memory traces are still fragile and vulnerable. Over the course of time, the information is assumed to become gradually integrated into the less forgetting long-term store, critically without overwriting older memories. The active system consolidation theory postulates that covert and repeated reactivations of newly encoded memories during slow wave sleep (SWS) sub-serve these integration processes.

Thus, it is assumed that the beneficial effects of sleep on memory consolidation rely on hippocampal memory reactivations during SWS, leading to memory reactivations in the neocortex and thereby promoting a stabilization and integration of those memories (Diekelmann & Born, 2010; Stickgold & Walker, 2013). Additionally, the active consolidation theory postulates that these beneficial effects of memory reactivations on memory are solely specific to sleep. Cholinergic activity, which is at a minimum during SWS, is assumed to enable the information transfer from the hippocampus to the neocortex by reducing the inhibition of hippocampal feedback neurons (Hasselmo, 2006). Conversely, the high acetylcholine level during wake is thought to block corresponding consolidation

processes. Accordingly, the strengthening and integrative effects of reactivation processes are supposed to be closely tied to the state of sleep.

Supporting the core assumptions of the active system consolidation theory, replay activity during sleep has been consistently reported in memory-related brain structures, particularly in the hippocampus, in rodents as well as in humans (O'Neill, Pleydell-Bouverie, Dupret, & Csicsvari, 2010; Pavlides & Winson, 1989; Peigneux et al., 2004; Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009). Wilson and McNaughton (1994) were able to demonstrate in a landmark study that multiple pairs of hippocampal place cells, which exhibited correlated activity due to their overlapping place fields during an exploration task, showed a highly similar correlation pattern of neuronal firing again during subsequent SWS. Further studies could demonstrate that not only the spatial but even the temporal pattern of neuronal firing that occur during exploration of a novel environment are reactivated in the same order during subsequent sleep (Skaggs & McNaughton, 1996). Inspired by these early findings a series of succeeding experiments could demonstrate the replay of hippocampal place cells during sleep, indicating that spontaneous memory reactivations are typically time-compressed by a factor of 10-20 (O'Neill et al., 2010) and are closely related to hippocampal sharp-wave ripples (SW-R) (Buzsáki, 2006), which represent the most prominent activity pattern in the hippocampus during Non-REM sleep. Furthermore, replay activity during sleep has not only been found in the hippocampus but as well in various other memory related brain regions (i.e., prefrontal cortex, ventral striatum etc. (Ji & Wilson, 2007a; C. M. A. Pennartz et al., 2004; Peyrache et al., 2009)), possibly mirroring the redistribution of memory representations.

Thus, summarizing there is ample evidence that newly encoded memories become spontaneously reactivated during sleep in the hippocampus as well as other memory related

areas, potentially underlying the beneficial effects of sleep in memory performance. Still, all these findings reviewed so far do not evidence the functional significance of reactivation processes for memory consolidation. The next section will address this issue in detail.

1.4 Inducing reactivations by cueing during sleep

Inspired by prior evidence implicating reactivations during sleep in memory formation, a first attempt to prove their functional significance for memory formation was made by Rasch and colleagues (Rasch, Büchel, Gais, & Born, 2007). In this study participants learned an object-location task while smelling a rose odor. During subsequent slow wave sleep, the odor was administered to some of the participant. Astonishingly, recall performance was enhanced in these subjects when compared to participants who did not receive the odor during SWS. Furthermore, functional magnetic imaging (fMRI) showed that odor presentation during SWS activated the hippocampus after learning. Thus, the exposure to the odor during sleep probably triggered reactivations of the learning context, which then benefitted associated memories. In a subsequent study Rudoy and colleagues (Rudoy, Voss, Westerberg, & Paller, 2009) were able to demonstrate that not only context cues such as odors are capable to induce reactivations during sleep, but even specific item cues such as individual sounds. In this study participants associated the locations of different cards with characteristic sounds. During succeeding sleep half of the sounds were replayed again to reactivate associated place-object associations. At later recall, memory performance for the reactivated associations was significantly better than for the non-reactivated associations. Furthermore, it could be demonstrated in a follow-up study that reactivation of the characteristic sounds was associated with increased activation in the right parahippocampal cortex (Dongen, Takashima, et al., 2012). Since then several studies could replicate the result

that externally cueing memories during sleep enhances memory performance (Diekelmann, Biggel, Rasch, & Born, 2012; Diekelmann, Büchel, Born, & Rasch, 2011; Rihm, Diekelmann, Born, & Rasch, 2014), while others extended these findings by showing that replay of prior learned melodies during sleep enhances skill learning (Antony, Gobel, O'Hare, Reber, & Paller, 2012; Schönauer, Geisler, & Gais, 2013), and promotes explicit knowledge of a motor task (Cousins, El-Deredy, Parkes, Hennies, & Lewis, 2014). Importantly, such beneficial effects of cueing during sleep depends on the structural integrity of the hippocampus, as demonstrated in epileptic patients with unilateral or bilateral hippocampal sclerosis (Fuentemilla et al., 2013). Thus all these studies provided precious insight into the memory function of sleep and its connection to memory reactivations.

Concerning the assumed sleep specificity, results obtained by cueing studies are not entirely clear. For olfactory cues, the beneficial effect of cueing appears to be sleep specific, as cueing during post learning wakefulness had either no or even detrimental effects on memory stability (Diekelmann et al., 2011; Rasch et al., 2007). For auditory cues, results are more ambiguous: while cueing of melodies during post-learning wakefulness did not improve memory (Schönauer et al., 2013), re-exposure to sounds during NREM sleep tended to improve memory for sound-place associations (Oudiette, Antony, Creery, & Paller, 2013; Rudoy et al., 2009). Critically, the former study was accomplished during night, while the latter experiments comprised naps in the afternoon. Hence, the divergent effects of auditory cueing during wakefulness might as well mirror the degree of the participants' tiredness.

This ambiguity with regards to the sleep specificity of cueing procedures goes hand in hand with findings concerning spontaneous reactivation processes. Signs of hippocampal replay and SW-R activity in rodents are also evident during wakefulness (Cheng & Frank,

2008; O'Neill et al., 2010) and spontaneous memory reactivations during the wake state were as well observed in humans (Peigneux et al., 2006). The active system consolidation theory postulates, as outlined above, that the cholinergic tone, varying between wakefulness and sleep, might lead to different consequences of hippocampal replay depending on the brain state. In line with these arguments, wake reactivations have been repeatedly related to recapitulation and anticipation of behavior, and less with the strengthening and transfer of newly encoded memories (but see (Dupret, O'Neill, Pleydell-Bouverie, & Csicsvari, 2010)). However, the role of wake reactivations for memory formation and their behavioural relevance is not entirely clear. As well, it is still an open question whether the benefits of auditory cueing on memory consolidation are solely sleep-specific or similarly occur to at least some degree during post-learning wakefulness.

Furthermore, while all of the studies reviewed above demonstrated the functional significance of memory reactivations during sleep and the possibility to externally influence those processes, it is not clear whether there exist boundaries when it comes to the stimulus material used to induce memory reactivations during sleep. While some studies used specific item cues (e.g. (Fuentemilla et al., 2013; Rudoy et al., 2009)), it still has to be determined whether even more complex cues such as linguistic material (i.e. words) are also capable of inducing reactivation processes.

Additionally, all of the studies reviewed so far have in common that they followed the assumption that memory cues presented during sleep should act as "reminder", thereby automatically triggering associated memory representations and finally strengthen those memory traces. This rationale has proved to be successful. However, whether additional input after each cue might improve or interfere with potential stabilization processes and

whether these effects are dependent on the type of input (e.g. congruent or incongruent word pairs) is as well unknown.

After having introduced the concepts of the active system consolidation theory and corresponding experimental data, the following section will describe the oscillatory phenomena, associated with memory formation during wakefulness and sleep.

1.5 Neural oscillations of memory formation during wakefulness and sleep

Concerning memory processes during wakefulness, extensive evidence has accumulated that memory encoding, maintenance and recall are heavily based on neural oscillatory synchronisation (Fell & Axmacher, 2011a). Specifically brain oscillations in the theta (4-7 Hz) and gamma (30-80 Hz) band have been consistently reported to be crucial for various processes associated with memory formation, be it memory encoding, the offline maintenance of memories or the retrieval of stored memories (for reviews see (Guderian & Düzel, 2005; Nyhus & Curran, 2010a)). For episodic long-term memory, increases in oscillatory theta power are consistently observed during successful encoding of information and predict later remembering of the newly learned information as reflected in the subsequent memory effect (SME) (Klimesch, 1999; Klimesch et al., 2006). These findings have led to the suggestion that oscillatory theta might represent the strength of a specific memory trace (Klimesch et al., 2006). Recent studies additionally report that the SME is associated with an increase in posterior gamma oscillations, as well as an increase in theta-gamma cross-frequency coupling. This has been suggested to allow an effective neural communication across distant brain areas (Siegel, Donner, & Engel, 2012) and the binding and temporal ordering of individual memories (Lisman & Jensen, 2013). However, while

these oscillatory mechanisms of memory formation have mainly been examined during wakefulness, it is unclear whether similar mechanisms accompany memory reactivations on a cortical level during sleep.

As stated above it is assumed that sleep specific memory reactivations in the hippocampus facilitate the gradual integration of memories from hippocampal into neocortical networks for long-term storage. A fine-tuned temporal relationship between neocortical slow oscillations, thalamo-cortical spindles and hippocampal sharp wave-ripples is suggested to coordinate this information flow between neocortical areas and the hippocampus during Non-REM sleep (Diekelmann & Born, 2010).

The <1 Hz slow oscillation represents the most prominent oscillation characterizing the electroencephalogram (EEG) during SWS (M. Steriade, 2006; Timofeev & Chauvette, 2011). The slow oscillation is primarily generated in the neocortex and mirrors global synchronous neural activity alternating between periods of membrane depolarization, accompanied by increased excitability ("up-states") and periods of hyperpolarization, associated with widespread neuronal quiescence ("down-state"), which spreads across the neocortex. Furthermore slow oscillations affect activity in other brain structures, such as the striatum, locus coeruleus and the hippocampus, potentially coordinating communication between these brain regions and the neocortex (Genzel, Kroes, Dresler, & Battaglia, 2014).

The active system consolidation theory ascribes the slow oscillation the role of a time giving pace maker, orchestrating neural processes related to the reactivation of prior encoded memories during sleep. It is suggested that the depolarizing up states of the slow oscillation drive repeated reactivations of memory representations in the hippocampus together with sharp wave ripples. Sharp waves are fast depolarizing bursts that become superimposed by ripple activity (100-300 Hz), representing the most prominent activity pattern in the

hippocampus during Non-REM sleep (Kudrimoti, Barnes, & McNaughton, 1999). Since slow oscillations drive at the same time thalamo-cortical spindles, they enable the formation of spindle-ripple events. Thus, individual ripple events and associated reactivated memory information become nested in individual spindle troughs (Siapas & Wilson, 1998), forming spindle-ripple events. These spindle-ripple events are assumed to provide the basis for the information transfer by which reactivated hippocampal memory information can trigger, via Ca^{2+} influx into neocortical pyramidal cells, enduring plastic changes underlying the long-term storage of information in neocortical areas (Born & Wilhelm, 2012).

Thus, summarizing, the theory postulates a crucial role of slow oscillatory activity in synchronizing hippocampal memory reactivations with thalamo-cortical spindle activity, thereby transferring and integrating the memories into long-term storage (Bergmann, Mölle, Diedrichs, Born, & Siebner, 2012; Dongen, Thielen, Takashima, & Barth, 2012; Rasch & Born, 2013; Rihm et al., 2014; Ritter, Strick, Bos, Van Baaren, & Dijksterhuis, 2012).

Up to now there is consistent but rather indirect experimental evidence concerning the assumptions of the model. In rodents, it has been demonstrated that reactivation activity, both in hippocampal and neocortical circuitry, emerges in the timeframe of the slow oscillation (Euston, Tatsuno, & McNaughton, 2007; Ji & Wilson, 2007b). Furthermore, selective disruption of SW-R's by electrical stimulation after learning, impairs the consolidation of acquired spatial memories in rats (Ego-Stengel & Wilson, 2010; Girardeau, Benchenane, Wiener, Buzsáki, & Zugaro, 2009), indicating a causal role of SW-R's for consolidation processes. Also, consistent with the concept, slow oscillations, spindle and hippocampal ripple activity are enhanced during sleep after learning (Eschenko, Ramadan, Mölle, Born, & Sara, 2008; Gais, Mölle, Helms, & Born, 2002a; Mölle, Eschenko, Gais, Sara, & Born, 2009). Specifically these increases seem to be associated with improved memory

performance after sleep (Clemens, Fabó, & Halász, 2005; Girardeau et al., 2009; Huber, Ghilardi, Massimini, & Tononi, 2004). While all these separate results fit to the predictions of the active system consolidation theory, the exact effects of reactivations during sleep especially in humans on the cortical level and its underlying oscillatory mechanisms remain to be identified.

After having introduced the concepts of the active system consolidation theory, the following sections will provide insight how sleep aids language learning and whether sensory events are processed during sleep. The latter issue is of particular interest with regards to the cueing literature, since those studies implicitly assume that external stimuli are detected and further processed during sleep. If and to what extend such processing takes place during sleep will be discussed in this section.

1.6 Sleep and language learning

Language is the mental ability, which we use to communicate. Thus, it is a quintessential human trait and fundamental for every-day life (Pinker, 2000). It comprises the association of symbols and sounds with meaningful concepts and allows us to describe the external environment and our internal thoughts. Language is thought to depend on two mental capacities: a memorized 'mental lexicon' and a computational 'mental grammar' (Chomsky, 1993; Pinker, 1994; M T Ullman, 2001). The mental lexicon includes all idiosyncratic, word- specific information. The principal types of memory concerning the mental lexicon are phonological (sounds of words), orthographic (spellings of words) and semantic (knowledge of the world) (Price, 2000). But language also comprises regularities. Those regularities can be captured by the rules of grammar. They determine how to combine lexical forms, in order to form complex representations and enable us to understand and

interpret such complex forms (Michael T. Ullman, 2004). Sleep seems to play a crucial role when it comes to language learning, by facilitating highly divers aspects, both with regards to the mental grammar and lexicon.

While most studies linking language learning and the memory function of sleep concentrated on the faculty of the mental lexicon and the learning and integration of new words, some recent evidence suggests that sleep is also essential in grammar learning. Three recent studies could demonstrate that sleep facilitates the learning and extraction of new grammatical rule (Batterink, Oudiette, Reber, & Paller, 2014; Gómez, Bootzin, & Nadel, 2006; Nieuwenhuis, Folia, Forkstam, Jensen, & Petersson, 2013). Specifically, infants who were allowed to sleep after exposure to an artificial grammar demonstrated greater rule abstraction (Gómez et al., 2006). In the same vein, in adults sleep enhances rule abstraction to an artificial grammar (Nieuwenhuis et al., 2013) and the learning of hidden linguistic rules (Batterink et al., 2014). In this context, the known beneficial effects of sleep for generalization and abstraction seem to be specifically important.

But as outlined above much more effort has been made in order to investigate the memory effects of sleep with regards to the mental lexicon. Numerous findings could evidence the beneficial influence of sleep on word learning. Sleep has been proven to benefit speech production (Gaskell et al., 2014), speech recognition (Fenn, Nusbaum, & Margoliash, 2003) and importantly the integration of new words into pre-existing knowledge networks (Dumay & Gaskell, 2007; Tamminen, Payne, Stickgold, Wamsley, & Gaskell, 2010). Furthermore, sleep has been shown to support vocabulary learning in children and adolescents (Gais et al., 2006; Henderson, Weighall, Brown, & Gareth Gaskell, 2012).

Theoretically, Davis and Gaskell (Davis & Gaskell, 2009a) proposed a model for word learning named the Distributed Cohort Model, which is closely linked to the active system consolidation theory and the standard two-stage model of memory consolidation. The model distinguishes two stages of learning and lexicalization of new words. The first stage comprises an initial episodic familiarization with the new words, which is thought to be accomplished by the hippocampus. Here, sparse individual representations are formed. At this stage the model proposes as well, that the newly encoded information is replayed off-line, particularly during sleep, which is thought to result in a strengthening of the distributed lexical representation (i.e.: phonological form and meaning of spoken words) in neocortical long-term memory. Through distribution, the lexical representation is allowed to compete with overlapping representations during speech perception (Davis & Gaskell, 2009b), enabling automaticity in word recognition. Thus, here too reactivation processes during sleep are assumed to play a crucial role, in this case particularly for word learning. While as reviewed above, ample evidence exists that sleep benefits the learning of new words, a more direct hint that the consolidation of words depends on covert reactivation processes during sleep is still outstanding.

While the last section introduced the connections of language learning and sleep, the upcoming section will examine how and to what extend the sleeping brain accomplishes sensory and linguistic processing.

1.7 Auditory processing and semantic discrimination during sleep

Traditionally it is assumed that the sleeping brain is shut off from the external world (Mircea Steriade & Timofeev, 2003), given that sleep is associated with a reversible reduction in behavioral responsiveness. Thus, activity in the cortex during sleep is supposed to be mainly internal (Braun et al., 1997), resulting from slow oscillations or other endogenous rhythms (M Steriade & Amzica, 2003). The mechanism which is thought to underlie this deprivation of the cortex from external sensory inputs is called 'thalamic gating' (McCormick & Bal, 1994). The term refers to the assumption that one major function of the thalamus seems to be the selective control of information flow during different brain states. It is suggested to represent the main processing hub where sensory signal transmission is attenuated during sleep (M Steriade, McCormick, & Sejnowski, 1993). During wake thalamo-cortical cells are in a tonic-depolarized state. This state allows excitatory postsynaptic potentials, triggered by incoming sensory impulses, to be easily transmitted to cortical areas (Coenen, 1998). During sleep, thalamo-cortical neurons experience massive hyperpolarization, which becomes apparent in the large amplitude, low frequency waves associated with Non-REM sleep. Originating from the brainstem, excitations in inhibitory thalamic neurons lead to thalamic relay neuron inhibition and further to a rebound excitation (McCormick & Bal, 1994). Thus, within phases of neural silence widespread neuron populations fire synchronously in bursts. This burst mode firing is thought to block a major part of the incoming sensory information at the thalamic level, since the incoming excitatory postsynaptic potentials cannot overcome the threshold caused by these hyperpolarizations (Coenen & Vendrik, 1972).

However, recent findings concerning the auditory system disagree with the notion

that the cortex is completely deafferentiated during sleep from sensory input, suggesting that the thalamus might not be as effective in gating sensory information. These studies could show that neurons of the primary auditory system are still responsive during sleep. Even more, responses during wakefulness and sleep seem to hardly differ (Edeline, Dutrieux, Manunta, & Hennevin, 2001; Issa & Wang, 2008; Nir, Vyazovskiy, Cirelli, Banks, & Tononi, 2013; Pena, Perez-Perera, Bouvier, & Velluti, 1999). Astonishingly, in a study by Issa & Wang (2008) neurons in the primary auditory cortex as well in the secondary auditory cortical areas displayed on average similar discharge patterns during wakefulness and sleep. Accordingly, the sleeping brain seems to be still responsive to external input, at least to auditory stimulation.

These findings are in line with research trying to assess the extend of external information processing during sleep, using event related potentials (ERP's). Several studies demonstrated that the sleeping brain is able to discriminate deviant auditory tones from repetitive ones during all sleep stages (for a review see, (Bastuji & García-Larrea, 1999)). Specifically during Non-REM sleep stage 2 and SWS deviant tones elicit K-complexes of higher amplitude. K-complexes are a specific type of slow events and can be triggered by sensory stimulation during sleep (Colrain, 2005). Some recent imaging experiments extended these findings by additionally accounting for the effects of ongoing neural activity on the brain's responses to auditory stimulation during sleep. Those studies, presenting tones during sleep, considered the appearance of the major spontaneous oscillations of Non-REM sleep, namely sleep spindles and slow waves, in their analysis. With regards to sleep spindles, Dang-Vu and colleagues (2011) showed that tones which were played outside the appearance of sleep spindles, activated the primary auditory cortex and thalamus, as well as some regions involved in the processing of auditory stimulation (cerebellum, middle frontal gyrus,

precuneus and posterior cingulate gyrus). Interestingly tones being played right into sleep spindles did not show any effect, neither in the thalamus nor in the auditory cortex. The authors concluded that sleep spindles reduce the transmission of sensory information to the cortex and might isolate the cortex from the environment. Thus, it seems that the thalamus per se does not deafferentiate the cortex globally from external input. Much more sleep spindles, which are known to have a thalamic origin seem to mediate specific time frames of cortical isolation. Furthermore, with regards to slow oscillations, the authors contrasted neural activity associated with tones that elicited K-complexes or did not. Tones, which elicited K-complexes, were associated with enhanced activation in the primary auditory cortex, suggesting that K-complexes are associated with enhanced processing of the stimulus at the cortical level, which is in line with earlier findings (Czisch et al., 2009).

While these studies demonstrated that at least a detection of physical characteristics might take place during sleep, further work using verbal material tried to assess whether the detection of the intrinsic meaning of a stimulus is still given during sleep. Two studies investigated the brains responsiveness to names, including the own name of the participants, during sleep (F Perrin, Garcia-Larrea, Mauguiere, & Bastuji, 1999; Pratt, Berlad, & Lavie, 1999). While all names evoked K-complexes during sleep stage 2, an early portion of the K-complex was selectively increased by the subject's name. These findings were bolstered by the results of a fMRI study, comparing the effects of presenting the subject's own or a pure tone during wakefulness and sleep (Portas et al., 2000). During wakefulness as well as during sleep stimulus presentation produced a similar pattern of activation, namely in the auditory cortex, thalamus and caudate. Furthermore, in both waking and stage 2 sleep, presentation of the subjects' names elicited stronger activation in the middle temporal gyrus and orbitofrontal cortex than tones did.

To further elucidate the brains ability for sematic discrimination during sleep using EEG, several studies concentrated on the so-called N400. This ERP component is elicited through the perception of semantically unrelated information or words and is understood as marker of verbal discordance. Astonishingly, presentation of semantically incongruent word pairs during sleep led to an enhanced N400 amplitude, suggesting that the sleeping brain is even able to perform semantic discrimination at least to some extend (Brualla, Romero, Serrano, & Valdizán, 1998a; Fabien Perrin, Bastuji, & Garcia-Larrea, 2002). Finally semantic discrimination during sleep was as well investigated using sentences (A. Ibáñez, López, & Cornejo, 2006). Sentences with different degrees of congruence were presented during sleep stage 2. As in wakefulness, the amplitude of the N400 varied according to the degree of congruency.

Thus, in contrast to the assumption that the cortex is isolated from external input during sleep, various studies could show that the sleeping brain is able to process auditory information. Moreover the capacity to establish neural representations of stimuli in sensory memory during sleep seems to be at least partly preserved. However, it is unknown whether processing of complex verbal cues during sleep is also capable of reactivating associated memories and thereby strengthening long-term memory formation.

1.7 Aim

While the notion that sleep favors memory formation due to reactivation processes during sleep has been established and growing evidence suggests that these reactivation processes can be influenced by cueing procedures, several important issues are still unknown. A major aim of this thesis was to extend the cueing of memories during sleep to linguistic material and to assess the neural activity associated with successful cueings during sleep. Thus, we investigated whether cueing of such highly complex material as foreign vocabulary during sleep would strengthen memory performance and whether we could identify neural patterns accompanying successful cueings. Additionally, we aimed at extending the rational of replaying single cues during sleep. We wondered whether additional input after each cue might improve or interfere with potential stabilization processes and whether these effects are dependent on the type of input. Therefor we investigating the behavioral and neural effects of replaying congruent (i.e. vocabulary pairs as learned before sleep) and incongruent (i.e. newly formed vocabulary pairs) word pairs during sleep. Furthermore, to assess the sleep specificity of potential cueing effects, we ran several wake control groups during nights as well as during daytime, in order to get a clearer picture of likely intervening influences such as tiredness.

Finally, as oscillatory theta activity during retrieval is suggested to represent the strength of a specific memory trace (Klimesch et al., 2006), we set out to investigate whether vocabulary cueing during sleep would alter theta power during subsequent recognition testing, thereby potentially indicating the memory strengthening effect of cueing on an oscillatory level.

1.8 Outline

Chapter 2 will determine whether foreign vocabulary is capable of inducing memory reactivations during sleep and describe neural patterns of successful reactivations during sleep (Boosting vocabulary learning by verbal cueing during sleep, Schreiner & Rasch, 2014, Cerebral Cortex)

Chapter 3 will investigate the effects of presenting prior learned Dutch German word pairs, with divergent congruence during sleep. Again associated neural activity will be described (When less is more: Auditory feedback blocks memory benefits of cueing during sleep**,** Schreiner & Rasch, 2015; submitted manuscript).

In **Chapter 4** a behavioral study is presented which particularly investigates the sleep specificity of auditory cueing procedures (Cueing vocabulary during daytime wake has no effect on memory, Schreiner & Rasch, 2015, under revision, Somnologie).

Chapter 5 will investigate whether signs of nighttime cueing are still detectable in subsequent theta activity associated with recognition memory (Cueing vocabulary during sleep increases theta activity during later recognition testing, Schreiner & Rasch, 2014, under revision, International Journal of Psychophysiology).

Chapter 6 will summarize and discuss the presented work.

TWO

Boosting vocabulary learning by verbal cueing during sleep

Thomas Schreiner
Björn Rasch

Cerebral Cortex, 2014
(advanced online publication)

Abstract

Reactivating memories during sleep by re-exposure to associated memory cues (e.g., odors or sounds) improves memory consolidation. Here, we tested for the first time whether verbal cueing during sleep can improve vocabulary learning. We cued prior learned Dutch words either during non-rapid eye movement sleep (NonREM) or during active or passive waking. Re-exposure to Dutch words during sleep improved later memory for the German translation of the cued words when compared with uncued words. Recall of uncued words was similar to an additional group receiving no verbal cues during sleep. Furthermore, verbal cueing failed to improve memory during active and passive waking. High-density electroencephalographic recordings revealed that successful verbal cueing during NonREM sleep is associated with a pronounced frontal negativity in event- related potentials, a higher frequency of frontal slow waves as well as a cueing-related increase in right frontal and left parietal oscillatory theta power. Our results indicate that verbal cues presented during NonREM sleep reactivate associated memories, and facilitate later recall of foreign vocabulary without impairing ongoing consolidation processes. Likewise, our oscillatory analysis suggests that both sleep-specific slow waves as well as theta oscillations (typically associated with successful memory encoding during wakefulness) might be involved in strengthening memories by cueing during sleep.

Introduction

Language acquisition is a quintessential human trait and fundamental for every-day communication (Pinker, 2000). Learning a new language depends essentially on the learning of new vocabulary, both for learning the native language as an infant as well as during acquisition of foreign languages in school children and adults (Shatz, 1999). It has been

suggested that sleep may play an important role in language learning (Davis & Gaskell, 2009a; Margoliash & Schmidt, 2010) possibly due to its beneficial role on memory consolidation (Rasch & Born, 2013). Sleep appears to facilitate memory for abstract relations of words of an artificial language in infants (Gómez et al., 2006) and benefits the integration of newly learned words into pre-existing knowledge in both school children and adults (Dumay & Gaskell, 2007; Henderson et al., 2012). More specifically, Gais et al. (2006) demonstrated that the ability of high school students to remember vocabulary of a foreign language was enhanced when learning was followed by sleep when compared with wakefulness.

According to the active system consolidation hypothesis, the beneficial role of sleep on language acquisition is due to a spontaneous and repeated reactivation of newly acquired information during subsequent non-rapid eye movement (NonREM) sleep, promoting memory stabilization and integration (Diekelmann & Born, 2010; Genzel et al., 2014; Stickgold & Walker, 2013). In support of the hypothesis, replay activity during sleep has been consistently reported in memory-related brain structures in rodents and humans, particularly in the hippocampus (O'Neill et al., 2010; Pavlides & Winson, 1989; Peyrache et al., 2009; Wilson & McNaughton, 1994) In animal models of language learning, reactivation of song patterns during sleep in birds is assumed to be critical for song learning during development (Dave & Marholiash, 2000), although mechanisms of memory consolidation during sleep may differ between mammals and birds, particularly with respect to system consolidation (Rattenborg, Martinez-Gonzalez, Roth, & Pravosudov, 2011). Furthermore, a series of recent studies has shown that experimentally inducing reactivations during NonREM sleep by using associated memory cues benefits memory consolidation using odors (Diekelmann et al., 2011; Rasch et al., 2007; Rihm et al., 2014; Ritter et al., 2012)

sounds (Dongen, Takashima, et al., 2012; Rudoy et al., 2009), or even melodies (Antony et al., 2012; Schönauer et al., 2013), including the successful cueing of hippocampal place cells during sleep in rodents (Bendor & Wilson, 2012a). In spite of the increasing evidence for the beneficial role of cueing during sleep on various memory processes (e.g., Oudiette and Paller, (2013), it remains an open question whether words can also be used as memory cues during sleep.

Based on studies using event-related potentials (ERPs), it has been suggested that the capacity to establish neural representations of stimuli in sensory memory during sleep is preserved (for a review, see Atienza et al., (2001)). For example, previous studies have shown that several ERP components (such as the auditory N1, the mismatch negativity, the P3a and 2 sleep-specific components, the N350 and the N550) react to a variable degree to different features of the stimuli presented during sleep, such as frequency and significance (e.g., the subjects' own name) (Brualla, Romero, Serrano, & Valdizán, 1998b; Fabien Perrin et al., 2002; Pratt et al., 1999) However, it is unknown whether processing of complex verbal cues during sleep is indeed capable of re- activating associated memories (e.g., the previously learned translation of the foreign word), thereby benefiting the consolidation of foreign vocabulary. Furthermore, it is still unclear whether cueing during sleep is purely beneficial or whether it is associated with "costs" by disturbing ongoing consolidation processes of uncued memories. Finally, the underlying event-related and oscillatory processes of successful reactivations during sleep are basically unknown.

In this study, we directly tested the hypothesis that verbal cueing during post-learning sleep enhances acquisition of foreign vocabulary. We hypothesized that cueing Dutch words specifically improves memory for cued words when compared with uncued words without disturbing consolidation of uncued words. Furthermore, we predict that the

improving effect of cueing is sleep-specific and does not occur after cueing during waking. In addition, we tested the hypothesis that event-related and oscillatory activity associated with cueing during sleep is predictive for cueing-related gains in vocabulary by recording high-density electroencephalography (EEG) during sleep.

Materials and Methods

Subjects

A total of 68 healthy, right-handed subjects (32 female, mean age = 24.61 ± 0.6) with German mother tongue and without Dutch language skills participated in the study. Seventeen subjects participated in each of the 4 experimental groups (e.g., main sleep group, control sleep group, active waking, and passive waking group). Four subjects had to be excluded from both sleep groups due to sleeping problems, resulting in 15 participants in each sleep group (main sleep group: 8 female, mean age = 25.1 ± 1.17 years; control sleep group: 8 female, mean age = 23.87 ± 0.68), 17 subjects in the active waking group (7 female, mean age = 24.7 ± 1.11 years), and 17 subjects in the passive waking group (8 female, mean age = 23.9 ± 0.97 years). Age and gender distribution did not differ between the experimental groups (both $P > 0.75$).

None of the participants were taking any medication at the time of the experiment and none had a history of any neurological or psychiatric disorders. All subjects reported a normal sleep-wake cycle and none had been on a night shift for at least 8 weeks before the experiment. Only subjects with a normal working memory capacity (i.e., minimum OSPAN score of 20, see task description, page 4) were recruited, due to the potential impact of working memory capacity on sleep-dependent declarative memory consolidation (Fenn & Hambrick, 2012). On experimental days, subjects were instructed to get up at 7.00 h and

were not allowed to take in caffeine and alcohol or to nap during daytime.

The study was approved by the ethics committee of the Department of Psychology, University of Zurich, and all subjects gave written informed consent prior to participating. After completing the whole experiment, participants received 120 swiss francs (CHF) (sleep groups) or 100 CHF (wake groups), respectively.

Design and Procedure

Participants entered the laboratory at 21.00 h. The session started with the application of the electrodes for standard polysomnography, including electroencephalographic (EEG; 128 channels, Electrical Geodesic, Inc.), electromyographic (EMG), and electrocardiographic (ECG) recordings. Prior to the experiment, participants of the sleep group spent an adaptation night in the sleep laboratory. In all 4 experimental groups, the learning phase started at ~22.00 h with the vocabulary learning task (Dutch–German word pairs, for a de- tailed description see Vocabulary Learning Task section). After completing the learning task, participants of both sleep groups went to bed at 23.00 h and were allowed to sleep for 3 h, whereas participants in the 2 wake control groups stayed awake (see Fig. 1, for an overview of the procedure). During the 3-h retention interval, a selection of the prior learned Dutch words was presented again during sleep stages N2 and N3 (slow wave sleep, SWS) in the cueing sleep group and during active or passive waking in the wake control groups for a total duration of 90 min (see below for a detailed description of the reactivation phase). In the control sleep group, the same procedure was administered but the selected Dutch words were not replayed during sleep. At ~2.00 h, subjects of both sleep groups were awakened from sleep stage 1 or 2 and at ~2.15 h, recall of the vocabulary was tested in all experimental groups.

Vocabulary Learning Task

The vocabulary learning task consisted of 120 Dutch words and their German translation, randomly presented in 3 learning rounds (word pairs are listed in the Supplementary Table 1). Dutch words were presented aurally (duration range 400–650 ms) via loudspeakers (70 dB sound pressure level). In the first learning round, each Dutch word was followed by a fixation cross (500 ms) and subsequently by a visual presentation of its German translation (2000 ms). The inter-trial interval between consecutive word pairs was 2000–2200 ms. The subjects were instructed to memorize as many word pairs as possible. In a second round, the Dutch words were presented again followed by a question mark (ranging up to 7 s in duration). The participants were instructed to vocalize the correct German word or to say, "next" (German translation: "weiter"). Afterward, the correct German translation was shown again for 2000 ms, irrespective of the correctness of the given answer. In the third learning round, the cued recall procedure was repeated without any feedback of the correct German translation. Recall performance of the third round (without feedback) was taken as pre-retention learning performance. In the third round, participants recalled on average 60.88 ± 1.1 words (range 40–82 words) of the 120 words correctly, indicating an ideal medium task difficulty (recall performance 50.41%) without any danger of ceiling or floor effects. We observed no difference in pre-retention memory performance between the 4 experimental groups (main effect of "condition": $F_{3,60} = 0.86$; $P = 0.46$), no difference in pre-sleep memory performance between later cued and uncued words (main effect "cueing": $F_{1,60} = 0.001$; $P = 0.96$) and interaction between condition and cueing ($F_{3,60} = 0.41$; $P = 0.74$; see for Table 1 for descriptive statistics).

Reactivation of Vocabulary

In the reactivation phase during the 3-h retention interval, Dutch words were presented aurally without the German translation. The presentation occurred via loudspeakers (50-dB sound pressure level). Of the 120 words learned before the retention interval, 60 words were cued and 60 were not cued during the subsequent retention interval. The 60 cued words consisted of 30 words that participants remembered during the pre-retention learning phase (cued hits), and 30 words that participants did not remember before the retention interval (cued misses). The words were individually and randomly chosen for each participant using an automatic MATLAB algorithm. In addition, 30 new words were presented during the retention interval that had not been included in the pre-retention learning list, serving as control stimuli. Thus, in total, 90 Dutch words were presented during the retention interval. Presentation occurred every 2.800–3.200ms in a randomized order for a total of 90 min, resulting in 10–11 exposures to each word (see Table 2). The rational of repeated cueing during sleep was derived from previous studies using olfactory cues which were repeated several times successfully induces memory reactivation during sleep (Diekelmann et al., 2011; Rasch et al., 2007; Rihm et al., 2014). Furthermore, we aimed at obtaining a sufficient number of trials for de- tailed EEG analysis. In the main sleep group, exposure to Dutch words occurred during sleep stages 2 and SWS. Sleep was continuously monitored by the experimenter, and the stimulation was interrupted when- ever polysomnographic signs of REM sleep, arousal, or awakenings occurred. On average, the presentation of Dutch words during sleep was interrupted 5.2 ± 0.5 times. In the control sleep group, Dutch words were also classified as "cued" and "uncued" words using the same procedure as in the main experiment, but the verbal cues were not administered during sleep. In the active waking group, cueing of Dutch words occurred during performance on a

computerized n-back task. The 3-h wake retention interval was divided into 30-min periods. In the first, third, and fifth 30-min period, participants performed on the n-back task (including a total of 27 67-s blocks of 0-back, 1-back, and 2-back blocks, in a randomized order, for more details see task description). Subjects were instructed to focus on the task and were given feedback on accuracy after each 30-min period. While subjects accomplished the n-back task, Dutch words were played in the same manner as in the sleep group, resulting in a total exposure time of 90 min. Between the 3 blocks of word reactivation, subjects completed questionnaires and played an online computer game (Bubble shooter). In the passive waking group, Dutch words were played during passive waking of the participants, allowing full attention on the replayed Dutch words. Participants were re-exposed to the Dutch words in the first, third, and fifth 30-min period of the 3-h retention interval. They were instructed that they would hear some of the Dutch words again and should attentively listen to the words. In the remaining 30-min periods, the participants performed on the n-back task and filled out questionnaires, without any auditory stimulation.

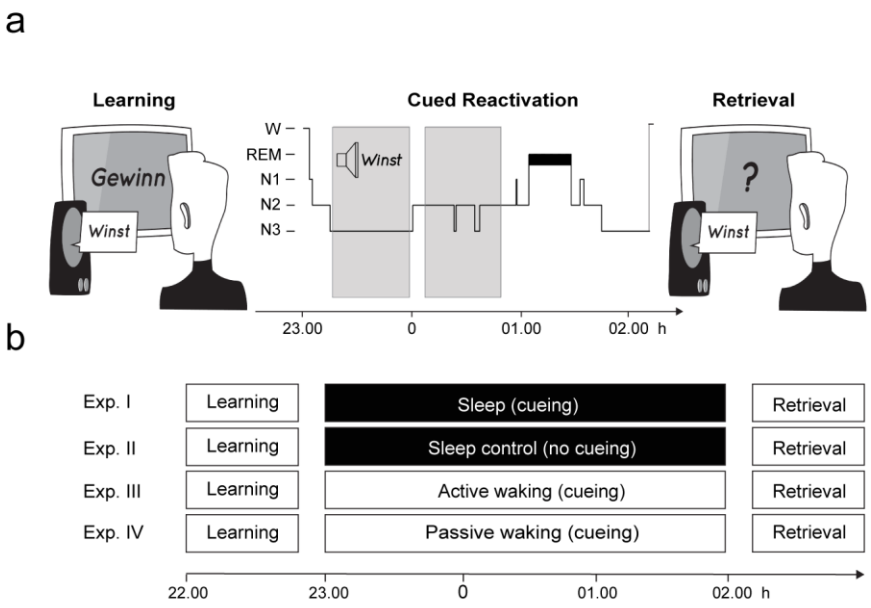
Recall of Vocabulary after the Retention Interval

During the recall phase, the Dutch words were presented aurally in a randomized order. In addition to the 120 words included in the pre-retention learning list, the 30 control words from the reactivation phase and 30 entirely new words were tested. After listening to the word, participants had to indicate whether the word was old (part of the learning material) or new. If the current word was recognized as old, they were asked to give the German translation.

As index of memory recall of German translations across the retention interval, we calculated the relative difference between the number of correctly recalled words before and

after the retention interval, with the pre-retention memory performance set to 100%. For recognition memory of Dutch words, we calculated the sensitivity index d′ [i.e., z(Hits) – z(False Alarms)] according to signal detection theory. Proportions of 0 and 1 were replaced by 1/2N and 1-1/2N, respectively, with N representing the number of trials in each proportion (i.e., N = 60, see Macmillan and Creelman, 82005)). The memory indices for cued recall and recognition were calculated separately for cued and uncued words.

Figure 1 Experimental Procedure



(**a + b**) Participants studied 120 Dutch-German word-pairs in the evening. Afterwards, participants of the main and the control sleep groups slept for 3 hours, whereas two other groups stayed awake. During the retention interval, 90 Dutch words (30 prior remembered, 30 prior not remembered and 30 new words) were repeatedly presented again. Cueing of vocabulary occurred either during NonREM sleep, during performance of a working memory task or during rest. The control sleep group did not receive any cues during sleep. After the retention interval, participants were tested on the German translation of the Dutch words using a cued recall procedure.

OSPAN Task

The OSPAN task was administered to assess the subjects' working memory capacity (Unsworth, Heitz, Schrock, & Engle, 2005). Each trial included an equation succeeded by a letter. The subjects had to indicate if the answer to a given equation was correct and had to remember the letter after- wards. Every 3–6 trials, 12 letters appeared on the screen and subjects had to select those that had been shown before.

n-Back Test

Subjects of both waking groups accomplished intermixed 0-, 1-, and 2-back versions of the n-back working memory task (Gevins & Smith, 2000). In this task, different letters appear successively in the center of the screen. In the 0-back version, subjects had to press a key whenever the letter "x" appeared on the screen. In the 1-back version, subjects had to respond to a letter repetition (h-f-f-k), while the 2-back version requires subjects to respond to a letter repetition with one intervening letter (h-f-s-f).

Sleep EEG

Sleep was recorded by standard polysomnography including EEG, EMG, and ECG recordings. EEG was recorded using a high-density 128-channel Geodesic Sensor Net (Electrical Geodesics, Eugene, OR, USA). High-density EEG was used to obtain a reliable estimation of possible topographical distributions to the reactivation-related effects. Impedances were kept below 50 kΩ. Voltage was sampled at 500 Hz and initially referenced to the vertex electrode (Cz). Additionally to the online identification of sleep stages, polysomnographic recordings were scored offline by 3 independent raters according to standard criteria (Iber et al. 2007). In order to exclude the possibility of sleep onsets in the

waking groups, EEG of the waking reactivation phase was also scored offline.

Event-Related Potentials

Offline EEG analysis was realized using Brain Vision Analyzer software (version: 2.0; Brain Products, Gilching, Germany). Data were re- referenced to averaged mastoids, low-pass filtered with a cutoff frequency of 30 Hz (roll-off 24 dB per octave), and high-pass filtered with a cutoff frequency of 0.1 Hz (roll-off 12 dB per octave). The EEG data were epoched into 1700ms segments beginning 200ms before stimulus onset. The 200ms interval preceding stimulus onset served as base- line and was used for baseline correction. Epochs were categorized based on performance between pre- and post-sleep tests yielding the following categories of ERPs: first, we analyzed ERPs for later remembered when compared with later forgotten cued words. In addition, we separated later remembered words in "Gains" (i.e., cued Dutch words not remembered before sleep but correctly recalled after sleep) and "HitHit" words (i.e., cued Dutch words remembered before and after sleep). Later forgotten words were separated in "Losses" (i.e., cued words correctly retrieved before sleep but not remembered after sleep) and "MissMiss" words (i.e., cued Dutch words not remembered before and after sleep). The control stimuli presented during the retention interval entered the category "Control". Signal averaging was carried out separately per subject and per condition and grand averages of all conditions were calculated. For statistical analysis, average EEG amplitudes measured over the interval from 800 to 1.100ms after stimulus onset were compared. To protect against error inflation due to multiple testing of multiple electrodes, we used a false discovery rate of $P < 0.05$. For illustration of the results, we present the ERP of the electrode with the highest significance (for sleep stage- specific ERP analyses, see Supplementary Results and Fig. 2).

Slow Oscillations Analysis

Artifact-free EEG data, ranging from −300 to 1500 ms with respect to the gain and loss trials, were low-pass filtered at 30 Hz and band-pass filtered between 0.5 and 4.0 Hz (stopband 0.1 and 10 Hz) using a Chebyshev Type II filter (MATLAB, The Math Works, Inc., Natick, MA, USA). Slow oscillations were then identified visually at electrode site Fz as well as electrode sites F3 and F4 as waves of a total duration >500ms and a minimal amplitude of 75 μV, starting in a time window between 0 and 800ms post-stimulus.

Analysis of Power Changes

We analyzed average power differences between Gains and Losses using a fast Frequency Transformation implemented in Brain Vision Analyzer with a Hanning Window of 10% during the 2.5 s after each word. Power values were analyzed for slow spindle activity (11–13 Hz) and fast spindle activity (13–15 Hz), as these frequency bands have been implicated in processes of memory consolidation (Antony et al., 2012; Cairney, Durrant, Hulleman, & Lewis, 2014; Fuentemilla et al., 2013; Rasch & Born, 2013) Frequency bands corresponding to slow wave activity (0.5–4 Hz) were not measured because of the limited number of possible cycles in the short trial length and border effects.

Theta oscillations (5–7Hz) were analyzed using a Continuous Wavelet Transformation as implemented in Brain Vision Analyzer (complex Morlet waveform, frequency range from 5 to 7 Hz in 10 logarithmic steps, Morlet parameter c = 7). In order to avoid edge effects, the trials entering the wavelet transform were segmented from −0.7 to 1.9 s with respect to stimulus presentation. An interval of 0.4 s at the beginning and the end of the trials was discarded afterward. A total of both induced and evoked activity was calculated by performing the wavelet analysis on single trials, after normalization with respect to the

prestimulus time window from −300 to −100ms (for the results of the total theta power calculation see Supplementary Fig. 1). Subsequently, the resulting single-trial frequency spectra were averaged. This procedure provides the overall power of a given frequency range. In order to obtain the induced power, which is thought to play a role in binding distributed cortical representations (Düzel, Neufang, & Heinze, 2005), we subtracted the theta effects of the average ERP (evoked power) from each single trial before calculating the time–frequency analysis and averaging the single trials. Statistical analysis was performed for a time window of 700–900ms after stimulus onset. Additionally, the same procedure was performed for slow spindles (11–13 Hz) and fast spindles (13–15 Hz), due to their assumed involvement in processes of sleep-dependent memory consolidation (for sleep stage-specific oscillatory analyses, see Supplementary Results and Fig. 3). As with the calculation of average oscillatory activity, frequency bands corresponding to slow wave activity (0.5–4 Hz) were not measured because of the limited number of possible cycles in the short trial length and border effects.

Statistical Analysis

Data were analyzed using repeated-measures analyses of variance (ANOVA). Where appropriate, significant interactions were further evaluated with Fisher's least significant difference post hoc tests. The level of significance was set to $P = 0.05$.

Results

Effects of Verbal Cueing on Memory for Dutch Vocabulary

As expected, re-exposure to Dutch words improved later memory for the German translation of the cued words, when cueing occurred during sleep. Participants correctly

recalled 105.14 ± 2.64% of the cued words, whereas only 95.43 ± 2.07% of the uncued words were remembered after sleep, with memory performance before sleep set to 100% (Fig. 2, see Table 1 for absolute values). The improvement of almost 10% points of vocabulary learning by cueing during sleep when compared with uncued words was highly significant ($t_{14} = 3.43$; $P = 0.004$). In fact, cueing during sleep even induced a 5% in- crease in memory for cued Dutch words above pre-sleep performance levels, and this increase reached a statistical trend (+5.14 ± 2.64%; $P = 0.072$, one-sample t-test, two-sided). In contrast, German translations of uncued Dutch words were significantly more forgotten when compared with recall performance before sleep (−4.75 ± 2.07%; $P = 0.045$). Thus, reactivation of vocabulary during sleep did not only prevent forgetting of German translations, but showed a trend of improving memory beyond baseline levels. On the individual level, 12 of 15 participants benefited from cueing (range +1 to +11 words, for the absolute difference between cued and uncued words), whereas 3 participants did not (range 0 to −1 words).

To test whether the observed benefits of cueing during sleep disturbed the consolidation of uncued words or not, we conducted an independent control experiment without presenting any verbal cues during sleep after learning (sleep control group). After learning, words were also classified as cued and uncued words using the same algorithm as in the main experiment (see Materials and Methods), but no verbal cues were replayed during sleep. As expected, recall of words classified as cued and uncued did not differ (93.55 ± 2.37 vs. 92.80 ± 3.10%; $t_{14} = 0.24$; $P = 0.81$). More importantly, memory performance in the sleep control group after sleeping without any verbal cues was highly comparable with the recall performance for uncued words observed in the main experiment with verbal cues during sleep (93.55 ± 2.37 vs. 95.43 ± 2.07%; $t_{14} = 0.71$; $P = 0.48$), and was significantly

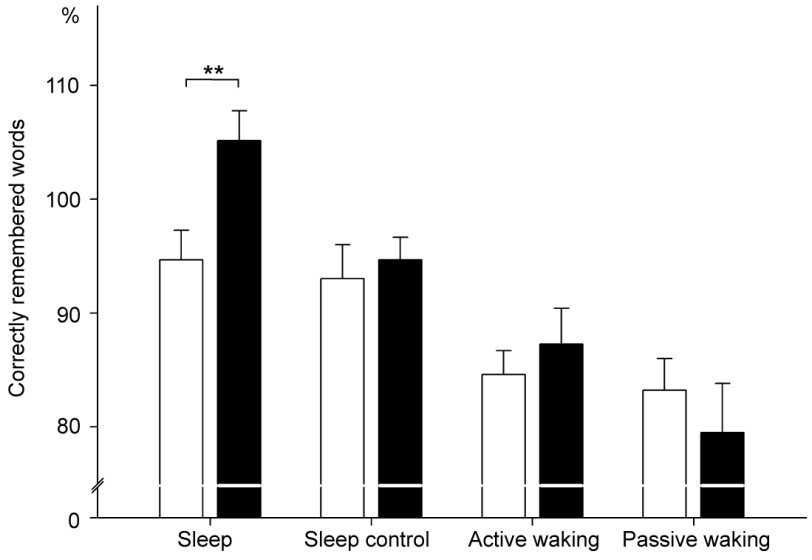
lower when com- pared with memory for cued words (92.80 ± 3.10 vs. 105.14 ± 2.64%; t_{14} = 3.26; P = 0.003, Fig. 2, see Table 1, for absolute values). In the 2 waking groups, cueing did not reveal any beneficial effect on memory for Dutch vocabulary, neither in the active waking group (85.53 ± 2.8 vs. 84.2 ± 2.16%, for cued and uncued words, respectively; t_{16} = 0.56; P = 0.58) nor in the passive waking group (79.86 ± 4.58 vs. 81.25 ± 2.09%), for cued and uncued words, respectively, t_{16} = −0.35, P = 0.74; see Table 1 for absolute values). Thus, even with the availability of attentive processing resources in the passive waking group, re-exposure to Dutch words during waking failed to improve memory for the German translations.

In addition to sleep-specific improvement by cueing, recall of German translation was generally better in the 2 sleep groups when compared with the 2 waking control groups, reflecting the well-known beneficial effect of retention intervals filled with sleep when compared with waking on memory consolidation (main effect condition; $F_{3,60}$ = 13.06; P < 0.001; see Fig. 2). Post hoc tests revealed that recall performance in both sleep groups independent of cueing was better when com- pared with the active waking and the passive waking group (t_{62} = 5.61; P < 0.001).

While cueing during sleep improved memory for German translation of Dutch words as tested by cued recall, we observed no sleep-specific benefit of cueing on recognition of Dutch words. The interaction remained non-significant ($F_{3,60}$ = 1.35; P = 0.15). However, sleep improved recognition of Dutch words independently of cueing (main effect condition; $F_{2,46}$ = 15.87, P < 0.001): both sleep groups showed a significantly higher recognition performance (main sleep group: d′=2.32±0.13; sleep control group: d′=2.04±0.14) when compared with the active waking group (d′ = 1.42 ± 0.16) and the passive waking group (d′ = 1.05 ± 0.16; all P < 0.001), while neither the 2 waking groups (P

= 0.10) nor the 2 sleep groups ($P = 0.68$) differed significantly among each other. In fact, recognition of cued and uncued Dutch words was basically identical in the main sleep group (see Table 1), safely excluding that recognition testing prior to cued recall might have confounded the reported beneficial effect of cueing during sleep as tested by cued recall. While cueing also did not affect recognition in the active waking group, cued words were better recognized in the passive waking group in an exploratory analysis, possibly reflecting the fact that the participants in the latter group at- tended the cued Dutch words during the retention interval (see Table 1).

Figure 2. Behavioral results



In the main sleep group, memory for cued word-pairs (black bar) was significantly improved as compared to uncued pairs (white bar). Recall of uncued word-pairs in the main sleep group was comparable to recall performance of word-pairs in the control sleep group, which did not receive any cues during sleep. No enhancing effects of cueing on later memory retrieval occurred in both waking control groups. Retrieval performance is indicated as percentage of recalled German translations with performance before sleep set to 100%. Values are mean ± s.e.m. $^{**}P \leq 0.01$.

Table 1. Overview of memory performance

		Cued	Uncued	*t*	*P*
Main sleep group					
Cued recall	*Learning*	29.87 ± 0.09	33.20 ± 2.54	-1.29	0.22
	Retrieval	31.40 ± 0.16	31.33 ± 2.17	0.04	0.97
	Change	+1.53 ± 0.79	-1.87 ± 0.70	3.52	0.003**
	% Change	105.15 ± 2.64	95.43 ± 2.07	3.43	0.004**
Recognition	*Hits*	52.40 ± 0.98	51.20 ± 1.57	1.33	0.80
	% Hits	87.33 ± 1.62	85.33 ± 2.62		
	d'	2.32 ± 0.15	2.32 ± 0.17	0.00	0.99
Control sleep group					
Cued recall	*Learning*	30	31.93 ± 1.84	-1.04	0.31
	Retrieval	28.07 ± 0.71	29.27 ± 1.66	-0.77	0.45
	Change	-1.93 ± 0.71	-2.66 ± 0.89	0.79	0.44
	% Change	93.55 ± 2.37	92.80 ± 3.10	0.24	0.81
Recognition	*Hits*	50 ± 1.24	50.60 ± 1.55	-0.64	0.53
	% Hits	83.33 ± 2.07	84.33 ± 2.59		
	d'	2.01 ± 0.13	2.09 ± 0.16	-0.93	0.36
Active waking group					
Cued recall	*Learning*	30.06 ± 0.10	30.59 ± 2.7	-0.19	0.89
	Retrieval	25.71 ± 0.83	26.12 ± 2.5	-0.19	0.85
	Change	-4.35 ± 0.84	-4.47 ± 0.63	0.12	0.90
	% Change	85.53 ± 2.81	84.21 ± 2.16	0.56	0.58
Recognition	*Hits*	50.29 ± 1.05	49.35 ± 1.55	0.79	0.43
	% Hits	83.83 ± 1.75	82.25 ± 2.59		
	d'	1.44 ± 0.15	1.39 ± 0.17	0.65	0.52
Passive waking group					
Cued recall	*Learning*	30.35 ± 0.14	27.82 ± 1.75	1.46	0.16
	Retrieval	24.24 ± 1.14	22.82 ± 1.78	1.17	0.25
	Change	-6.11 ± 1.41	-5.00 ± 0.59	-0.79	0.44
	% Change	79.86 ± 4.58	81.25 ± 2.09	-0.35	0.74
Recognition	*Hits*	46.53 ± 1.83	43.71 ± 1.85	2.88	0.01*
	% Hits	77.54 ± 3.06	72.84 ± 3.08		
	d'	1.13 ± 0.17	0.95 ± 0.17	2.41	0.02*

Data are means ± s.e.m; Numbers indicate absolute or relative values of correctly recalled or recognized words that where presented during the retention interval (cued words, 60 in total) or not (uncued words, 60 in total). For cued recall testing, number of correctly recalled words during the learning phase before and the retrieval phase after the retention interval are indicated. Change (% Change) refers to the absolute (relative) difference in performance between learning and retrieval phases. Hits (%Hits) refers to the absolute (relative) number of correctly recognized words as "old" (since %Hits = Hits*100/60, statistics are redundant). The sensitivity measure d' reflects recognition performance according to signal detection theory based on the proportion of Hits and False Alarms (Macmillan & Creelman, 2005) . *$P < 0.05$; ** $P < 0.01$.

Sleep and Cueing

The beneficial effect of cueing on memory during NonREM sleep cannot be explained by general alterations in sleep as the effect was specific for cued when compared with uncued words, while the general improving effect of sleep on memory was present for both word categories. Sleep architecture was not altered by cueing, as sleep parameters recorded in the main sleep group did not differ from those of the control sleep group (see Table 2). In addition, we did not observe any in- creases in alpha power 1000ms before (indicative of brief awakenings (Rudoy et al., 2009)) and after the auditory stimulation at electrode site Oz, excluding that cueing of words induced short lasting arousal responses (alpha power before (2.12 ± 0.41 μV) and after the auditory cue (2.01 ± 0.5 μV), respectively, $t_{14} = 0.31$, $P = 0.75$). Still, participants of the main sleep group spent more time awake then subjects of the control sleep group (4.66 vs. 0.55 min; $t_{14} = 2.86$, $P = 0.013$), indicating that auditory cueing slightly interrupted sleep. Note that auditory presentation of words was stop whenever signs of arousal or awakenings were detected. Importantly, performance levels of uncued words in the main sleep group and in the sleep group without cueing were almost identical, indicating that increases in wake time did not impair ongoing and spontaneous processes of memory consolidation. We did not observe any significant associations between the memory advantage induced by cueing (i.e., by subtracting memory for cued minus uncued words (Antony et al., 2012)) and the relative time spent in a certain sleep stage (N1: r = 0.18, $P = 0.50$; N2: r=−0.360, $P = 0.18$; SWS: r = 0.18, $P = 0.51$; REM: r = 0.24, $P = 0.93$). Cueing was monitored online and was restricted to sleep stages N2 and SWS. The total number of cueings did not differ between N2 and SWS (Table 2), and we did not observe any significant association between the memory advantage induced by cueing and number of cueings inN2 or SWS (N2: r = −0.39, $P = 0.14$; SWS: r = 0.1, $P = 0.72$; for a

more detailed description and analysis see Supplementary Table 2 and Results). Additionally, EEG offline scoring of the waking groups revealed no signs of sleep onsets, indicating that the subjects of both waking groups were awake throughout the reactivation phase.

Table 2. Sleep and reactivation parameter

	Main sleep group	Control sleep group	*P*
Duration [min]			
N 1	7.76 ± 1.66	5.20 ± 1.46	0.16
N 2	93.16 ± 5.93	100.27 ± 4.71	0.71
SWS	62.26 ± 5.8	57.93 ± 5.37	0.94
REM	22.13 ± 3.18	22.07 ± 2.73	0.37
WASO	4.66 ± 1.71	0.37 ± 0.14	0.03
Duration [%]			
N 1	4.02 ± 0.84	2.72 ± 0.70	0.31
N 2	48.70 ± 2.64	53.73 ± 2.95	0.25
SWS	33.11 ± 3.26	31.13 ± 2.95	0.72
REM	11.38 ± 1.59	11.65 ± 1.36	0.89
WASO	2.35 ± 0.82	0.002 ± 0.00	0.01
Number of reactivations			
N 2	442.86 ± 40.68	-	
SWS	508.80 ± 54.42	-	

Data are means ± s.e.m. N1, N2: NonREM sleep stages N1 & N2, SWS: slow-wave sleep / N3, REM: rapid eye movement sleep, WASO: wake after sleep onset.

Neural Correlates of Cueing During Sleep

In order to characterize the process of cueing on a neural basis, we analyzed ERPs and oscillatory responses to vocabulary cues during sleep. First, we analyzed ERPs for later remembered when compared with later forgotten cued words. In addition, we separated later remembered words in Gains (i. e., cued Dutch words not remembered before sleep but correctly recalled after sleep) and HitHit words (i.e., cued Dutch words remembered before and after sleep). Later forgotten words were separated in Losses (i.e., cued words correctly

retrieved before sleep but not remembered after sleep) and MissMiss words (i.e., cued Dutch words not remembered before and after sleep). Please note that the categories Gains and Losses reflect a clear behavioral change after cueing, therefore best representing the neural pattern associated with processes underlying successful versus unsuccessful cueing for later memory retrieval. In contrast, neural correlates of HitHit and MissMiss words are more difficult to interpret, as cueing during sleep might be ineffective for sufficiently strong memory traces (cases of HitHit) or non-existing associations (cases of "LossLoss") after encoding before sleep (for the behavioral analysis of Gains and Losses please see Supplementary Results and Table 2).

Remarkably, the EEG analysis of the average ERP amplitudes in the main sleep group clearly revealed a more pronounced negativity for subsequently remembered versus subsequently forgotten cued words at electrode site Fz ($t_{14} = -2.85$, $P = 0.013$). We further explored this difference by separately analyzing Gains and "HitHits" as well as Losses and MissMiss. Similar to the previous analysis, the difference between the ERP responses associated with HitHits when compared with "MissMisses" was significant ($t_{14} = 2.45$, $P = 0.028$). More importantly, we observed the largest negative amplitude associated with cueing of "Gain" words. Neural correlates of Gains represent a memory gain induced by cueing during sleep (i.e., successful verbal cueing during sleep), and the amplitude was significantly increased when compared with all other word categories at electrode site Fz in a time interval from 800 to 1100ms after word onset ($F_{6,84} = 4.52$, $P = 0.001$), all pairwise post hoc tests $P < 0.04$, see Fig. 3a,b). As Losses are the most suitable control category for Gains (i.e., behavioral change in memory induced by cueing, relatively similar number of occurrences, etc.), we focused on the comparison between Gains and Losses in all subsequent analyses. The analysis of all electrode revealed that the amplitude difference between Gains and

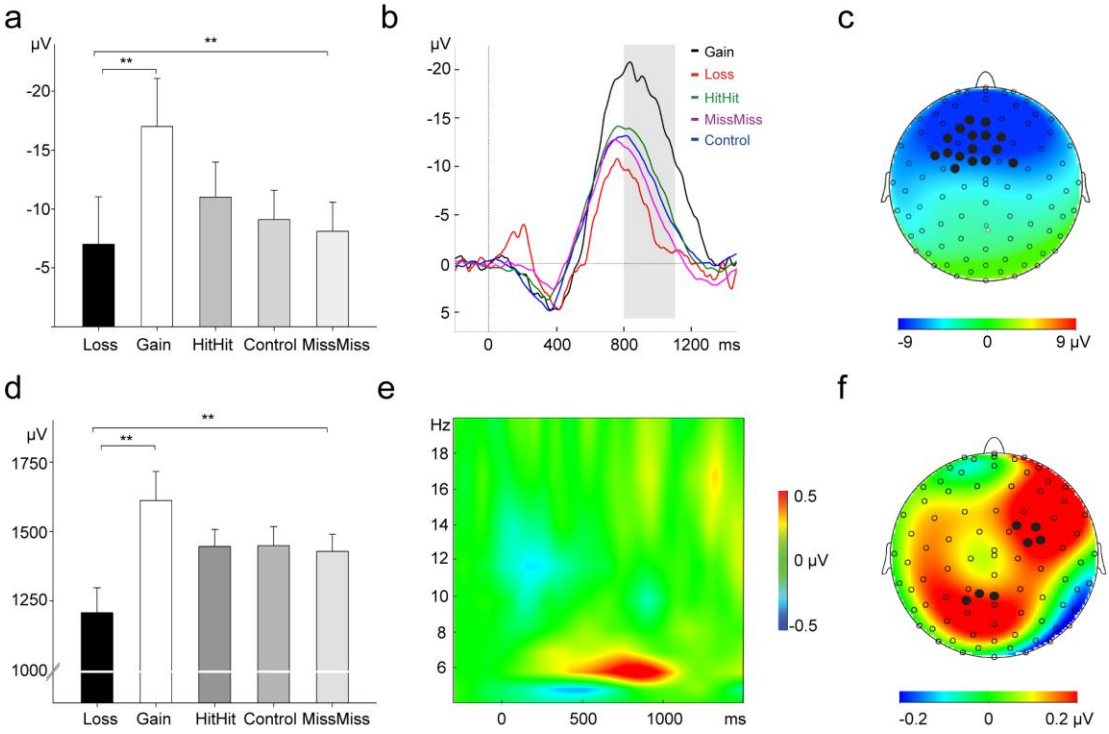
Losses had a stable fronto-central distribution (see Fig. 3c) comparable with distributions of sub- sequent memory effects observed during waking (Werkle-Bergner, Müller, Li, & Lindenberger, 2006). Furthermore, in a single-trial analysis, we counted the number of clearly identifiable slow waves (negative amplitude >75 μV with a duration of >500ms starting in a time window 0–800ms post-stimulus, see Materials and Methods) that followed cueing of Gain words when compared with Losses during sleep. This analysis revealed, that Gains were significantly more often followed by slow oscillations (31.09 ± 3.6% of all cueing trials of Gains) when compared with Losses (18.48 ± 3.4% cueing trials of Losses; $t_{14} = 5.35$, $P < 0.001$). This result was found at electrode site Fz, as well as F3 and F4 indicating a stable frontal distribution of this effect. This result is compatible with the assumption that the presence of a slow oscillation after the presentation of a Dutch word during sleep plays an important role for successfully stabilizing the associated memory trace, reactivated by the memory cue presented during sleep. As both slow oscillations and sleep spindles are critically involved in processes of memory consolidation during sleep (Rasch & Born, 2013) we also analyzed possible differences in average oscillatory power between Gains and Losses for slow spindles (11–13Hz) and fast spindle activity (13–15 Hz). However, we did not observe any difference between Gains and Losses in this analysis (all $P > 0.10$).

We further explored difference between Gains and Losses in time–frequency space. We controlled for a possible contribution of the evoked brain response by subtracting the average ERP (evoked power) from each single trial before calculating the time–frequency analysis (induced power) (Klimesch, Russegger, Doppelmayr, & Pachinger, 1998). In contrast to our expectations, the time– frequency analysis revealed no significant increase in oscillatory power in the spindle band related to Gains versus Losses, neither in the fast spindle band (13–15 Hz) nor in the slow spindle band (11–13 Hz). However, sleep stage-

specific analyses revealed a significant increase in slow spindle power during SWS (but not during stage N2) in a time window 600– 800ms after the cue ($P < 0.05$, for details see Supplementary Results and Fig. 3). Please note that the analysis of power changes in the slow oscillations/delta band was not possible due to the relatively small inter-trial interval between verbal cues.

Finally, we also analyzed power changes for the theta band. Theta activity is prevalently linked to successful memory encoding during waking (Nyhus & Curran, 2010a) and post-stimulus increases in induced theta power have been specifically linked to processes of recollection (Düzel et al., 2005). Interestingly, induced theta power associated with verbal cueing during sleep differed significantly between conditions ($F_{4,56} = 7.38$, $P = 0.002$). Gains were associated with an increase in induced theta power in a time window of 700–900 ms after stimulus onset. The increase in induced theta power was particularly strong in right frontal as well as left parietal electrodes (e.g., electrode FC6: $t_{14} = 3.68$; $P = 0.009$), strongly suggesting that a transient increase in theta power is critical for successful cueing during sleep (see Fig. 3d–f; see Supplementary Fig. 1 for total power changes). Interestingly, increases in theta activity for Gains when compared with Losses were more pronounced during stage 2 sleep, but were also reliably observed during SWS (see Supplementary Results and Fig. 3).

Figure 3. Electrophysiological results



ERPs and oscillatory theta power recorded during cueing in the sleep group were computed for words, for which cueing during sleep led to a change in memory performance. "Gains" reflect cued words not remembered in the pre-sleep test but correctly recalled in the post-sleep test. "Losses" refer to cued words remembered in the pre-sleep test but not in the post-sleep test. Words remembered before and after the retention interval were labeled "HitHit" and words not remembered both before and after the retention interval were labeled "MissMiss". The new 30 Dutch words formed the "Control" condition. (**a + b**) Successful cueing was associated with a more pronounced negativity at frontal electrode sites (representative electrode Fz). The rectangle illustrates the time window used for waveform quantification. (**c**) Scalp map representing the topographical distribution for the difference between "Gains" and "Losses" in the time window between 800 and 1100 ms, indicating a pronounced frontal distribution (all electrodes entered the analysis; black dots indicate significant electrodes at $P < 0.05$, false-discovery rate (FDR) corrected for multiple comparisons). The following electrodes were significant: E4, E5, E6, E11, E12, E13, E16, E19, E20, E23, E24, E28, E29, E35 E112 (see Supplementary Fig. 2 for the exact electrode positions). (**d + e**) Induced theta power for the difference between "Gains" and "Losses" (electrode FC6), indicating a distinct increase in induced theta power associated with successful cueing. (**f**) Scalp map depicting the distribution of theta power increase for "Gains" relative to "Losses" in the time window between 700 and 900 ms. The following electrodes were significant: E53, E60, E61, E62, E111, E117 (FC6), E118). $^{**}P \leq 0.01$.

Discussion

Our findings show for the first time that cueing prior learned foreign vocabulary during sleep improves later recall. Further- more, memory performance for uncued words in the main sleep group resembled memory performance of participants who did not receive any verbal cues during sleep, suggesting that cueing led to a real gain in memory performance. In addition, successful cueing during sleep, which resulted in later memory gains during retrieval testing, was associated with an increased late negativity and increased theta activity during NonREM sleep.

The beneficial effect of cueing during sleep is consistent with the active system consolidation hypothesis, which assumes that spontaneous memory reactivations during sleep are critical for the enhancing effect of sleep on memory consolidation. In fact, recent studies have successfully used memory-associated odors, sounds, or melodies (Antony et al., 2012; Rasch et al., 2007; Rudoy et al., 2009) to cue and strengthen memories during sleep. Here, we go an important step beyond these previous results by showing that also complex stimuli like foreign vocabulary can be successfully used to reactivate memories during leep, leading to an enhanced memory for vocabulary the next day. Importantly, our results are highly relevant for vocabulary learning in an educational setting, because our procedure of re- activating foreign vocabulary could be easily applied to these every-day learning contexts. However, as retrieval was tested in the night after only a few hours of sleep in the current study, future studies should test the memory-improving effects of cueing during sleep the next day or after several days. In addition, it still needs to be determined whether or not the beneficial effects of cueing during sleep are possibly accompanied by any detrimental effects on sleep-dependent memory consolidation of other material learned during the day. Finally, future studies need to examine whether cueing of vocabulary during sleep indeed facilitates

foreign language learning.

In our experiment, we explicitly chose Dutch as a foreign language to achieve sufficiently few learning trials required for our analysis. Due to the close relation of Dutch to German or English, German-speaking participants could more easily learn the vocabulary and might even be able to correctly guess the meaning of some words. However, guesses cannot explain our reported improved effect of cueing during sleep, as words were randomly assigned to the cued and uncued conditions. Furthermore, we can exclude that cueing simply increased perceptual fluency (Jacoby & Dallas, 1981) because mere exposure to the words during waking similarly increases perceptual fluency and had no effect on memory for the vocabulary in our study. Still, the degree of prior knowledge of related languages, learning difficulty, and memory strength during encoding might be important factors determining the effectiveness of cueing during sleep, requiring further examination. Most importantly, the close relationship of the languages Dutch and German might have considerably affected the successful effect of cueing during sleep in our study. Thus, replicating our results with more distant languages is necessary to generalize our findings.

In contrast to the beneficial effect of cueing during sleep on recall of German translations, recognition of Dutch words was not affected by cueing during sleep. This result suggests that cueing during sleep specifically strengthens the association between the Dutch words and the German translations in memory, thereby facilitating later recall. However, recognition was only tested once (and not before and after the retention interval), which might have reduced the sensitivity of this test for possible beneficial effects of cueing during sleep on memory consolidation. Importantly, the null effect on recognition safely excludes that the reported beneficial effect of cueing during sleep on later recall might be confounded by prior recognition testing or higher familiarity with the cued words. Interestingly, sleep in

general (independent of cueing) improved both recognition of Dutch words and recall of German translations, suggesting a broader role of sleep in memory consolidation when compared with experimental cueing during sleep.

Moreover, our results provide first evidence that the beneficial effects of cueing during sleep exceed the normal consolidation effects of sleep on memory, since recall of uncued words in the main sleep group was almost identical to memory performance of sleeping control participants who did not receive any cues during sleep. Thus, verbal cueing during sleep appears to benefit later recall of cued memory associations without disturbing ongoing consolidation processes during sleep. Hence, from a behavioral level, it appears as if the beneficial effect of cueing during sleep on memory occurs without any obvious costs. However, future studies in animal models or using intracranial recordings might additionally examine, in order to get a more comprehensive view, whether verbal cueing during sleep does not interfere with ongoing reactivation and consolidation processes also on the neural level. In contrast to our finding for verbal cues, others (Antony et al., 2012; Schönauer et al., 2013) reported some evidence for costs of cueing of procedural memories during sleep, as performance on the uncued sequence after receiving cues during sleep was lower when compared with performance in a separate group which did not receive any cues during sleep. Also here, future studies need to determine the mechanisms underlying a potential biasing of consolidation processes of cueing procedural memories during sleep when compared with the benefits of verbal cueing during sleep.

In the wake groups, the lack of beneficial memory effects by cueing was independent of the availability of attentional re- sources: both unattended cueing (active wake group) as well as attended cueing (passive wake group) during wakefulness failed to improve later retrieval of cued words. Thus, even though several rodent studies have reported the

existence of spontaneous replay activity during periods of quiet (passive) waking (Gerrard, Kudrimoti, McNaughton, & Barnes, 1986; Kudrimoti et al., 1999) it may not serve the same function as replay during NonREM sleep, as inducing reactivation during this behavioral state does not improve memory at least in humans. The lack of a memory effect by cueing during wakefulness is well in line with recent findings emphasizing the critical role of active and effortful retrieval to strengthen memories during wakefulness, whereas pure repeated study of words (without active retrieval testing) is not sufficient to improve memory (Karpicke & Roediger, 2008). Please note that cued words were played rather fast in our study (one word every 3 s), possibly not leaving enough time for active retrieval attempts.

Still, our results concerning the sleep specificity and the lack of beneficial effects of cueing in the waking groups should be interpreted with caution, because reactivation in both wake groups occurred during the night (11.00–02.00 AM) to exclude circadian factors on learning and retrieval. Thus, tiredness by partial sleep deprivation might have influenced the effects of cueing on memory performance. However, young participants (and particularly students) are typically quite used to stay up until 2.00 AM on weekends, so we consider the possible impact of tiredness on memory performance in the wake groups to be rather small. Furthermore, even if testing participants in the afternoon would result in a beneficial effect of cueing on memory, one could speculate that the underlying processes of this advantage are different from those acting during sleep: partial sleep deprivation mostly affects prefrontal functions like attention, working memory and possibly also task-related motivation. These processes are apparently not relevant for the benefits of cueing during sleep. One might hypothesize that cueing during sleep appears to benefit memory consolidation in an automatic, effortless und involuntary way, whereas benefits of cueing during wakefulness might possibly depend on the avail- ability of attentional resources, high

motivation, and active re- encoding of cued words. In contrast to this hypothesis, a recent study demonstrated beneficial effects of cueing in the afternoon during performance of a working memory task (Oudiette et al., 2013), possibly suggesting that cueing during wakefulness might improve memory even in the absence of attentional resources. Thus, an alternative explanation could be that the beneficial effects of cueing during wakefulness depend on an optimal circadian time, and that cues delivered during wakefulness at night-time cannot be successfully processed as the brain is already overloaded by information encoded during prolonged prior wakefulness. As the memory mechanisms underlying cueing during wakefulness are still unclear, further investigation regarding the sleep specificity of cueing benefits are clearly needed.

In contrast to previous reactivation studies, we administered reactivation cues during both N2 sleep and SWS instead of restricting reactivation to SWS. The rational for including N2 sleep was that 1) reactivation studies in rats do not differentiate between N2 sleep and SWS and 2) no previous reactivation study in humans has explicitly tested the effects of reactivation during N2 sleep on memory. Thus, we included N2 to obtain more time for repeated reactivation of Dutch words. In our view, early N2 sleep and SWS differ rather quantitatively (with respect to the occurrence of slow oscillations) than qualitatively, and our results suggest that cueing during N2 sleep might have at least no detrimental effects or even support memory consolidation during sleep.

In accordance to the active system consolidation, which assumes a critical role of slow oscillatory activity in synchronizing hippocampal memory reactivations with thalamo-cortical spindle activity (Bergmann et al., 2012; Dongen, Takashima, et al., 2012; Oudiette et al., 2013; Rasch & Born, 2013; Rihm et al., 2014; Ritter et al., 2012) successful cueing in our study was accompanied by an in- creased number of post-stimulus slow oscillations.

However, and in contrast to our expectations, this difference was not accompanied by an increase in sleep spindle activity, when analyzing sleep stage N2 and SWS together. Interestingly, the SWS-specific analysis revealed enhanced oscillatory power in the slow spindle band (11–13 Hz) succeeding the replay of Gains with regards to Losses. Both slow and fast sleep spindles have been related to memory improvement (e.g., Schabus et al. (2008)), while some recent study claimed that especially slow spindles during SWS seem to play a crucial role for memory consolidation (Cox, Hofman, & Talamini, 2012), which led the authors to suggest that the possible potentiating effects of spindles for memory consolidation are tied to their co-occurrence with slow oscillations. This interpretation would fit to our data, since successful cueing was, as mentioned above, accompanied by an increased number of post-stimulus slow oscillations as well as an enhanced oscillatory power in the slow spindle band.

Slow oscillations have been shown to play a causal role in processes of declarative memory consolidation during sleep (Marshall, Helgadóttir, Mölle, & Born, 2006; Ngo, Martinetz, Born, & Mölle, 2013) and might therefore also provide an important temporal time frame for stabilizing and consolidating externally induced memory reactivations by verbal cueing. To further examine the exact temporal relation- ships between verbal cueing during sleep and slow oscillations, future studies will need to systematically vary the onset of verbal cues presented during sleep in accordance to the up and down states of the ongoing slow oscillations.

Additionally, the results of the EEG time–frequency analysis indicate that successful cueing during sleep (i.e., cueings leading to enhanced memory performance) is accompanied by post-stimulus increase in induced theta power at right frontal and left parietal regions. Induced theta during waking has been linked to the encoding and retrieval of new declarative

information (Klimesch, 1999; Nyhus & Curran, 2010a). In addition, theta oscillations have been suggested to play a functional role in controlling, maintaining and storing memory content during wakefulness (Nyhus and Curran, 2010; Lisman and Jensen, 2013for reviews). During sleep, ongoing theta rhythms have been mainly associated with hippocampal activity during REM sleep, whereas the role of theta activity during NonREM sleep is less clear (Cantero et al., 2003). However, some recent studies have indeed implicated theta activity during NonREM sleep in processes of memory consolidation. Faster theta frequency or increased theta power during NonREM sleep predicted better subsequent memory performance in patients with Alzheimer's disease or amnestic mild cognitive impairment (Hot et al., 2011; Westerberg et al., 2012). Schabus et al. (2005) observed a similar results pattern in healthy subjects, leading to the author's speculation that in- creased theta activity during NonREM sleep might be associated with the reactivation of newly encoded information and as a consequence with improved memory performance. Our results partly support this notion emphasizing the importance of increases in theta power after reactivation for successful memory consolidation during sleep. However, whether these processes observed during sleep are indeed similar to theta in- creases underlying successful memory encoding during wakefulness and whether or how they relate to hippocampal theta rhythms require further examination. In general, the results reported here also indicate that complex auditory cues like foreign vocabulary are indeed capable of reactivating associated memories during sleep, suggesting that some processing of the presented words is pre- served during sleep (at least to some extent). Similarly, previous studies presenting verbal material during sleep have suggested a preserved capacity to discriminate semantic incongruency as well as the participants own name from other names during sleep (Brualla et al., 1998b; A. Ibáñez et al., 2006; F Perrin et al., 1999; Pratt et al., 1999). The successful

reactivation of memories during NonREM sleep was accompanied by an in- creased negativity over frontal brain regions, resulting in improved retrieval after sleep. The observed time interval, as well as the frontal topography associated with this "subsequent re-activation effect," is similar to ERPs typically observed during encoding for later remember items (i.e., the subsequent memory effect). In particular, an increased negativity has been reported during encoding of subsequently remembered stimuli using auditory presentations (Cycowicz & Friedman, 1999; Guo, Voss, & Paller, 2005), whereas subsequent memory for visually presented items is typically accompanied by more positive going ERPs in prefrontal and medio-temporal regions (Friedman & Johnson, 2000; Werkle-Bergner et al., 2006). In spite of these morphological similarities, it remains an open question whether neural generators and mechanisms underlying the subsequent reactivation effect observed during sleep are indeed similar to processes underlying encoding and retrieval during wakefulness.

To better understand the underlying function of the reported enhanced late negativity associated with successful cueing during sleep, we can only refer to studies using auditory stimuli to investigate the extent of information processing during sleep. Some of those studies focused on the formation of stimulus representations in sensory memory by performing different kinds of oddball paradigms (for a review see Atienza et al., 2001). In a study by Niiyama et al. (1995), participants were trained to react to rare sound stimuli during wake. Re- exposure to rare sounds during sleep stage N2 was associated with an enhanced late negativity over frontal electrodes (labeled as N350 and N550) when compared with frequent tones. The authors interpreted this component as part of elicited K-complexes, which might reflect a certain level of which might reflect a certain level of information processing. In a similar oddball study (Karakaş, Cakmak, Bekçi, & Aydin, 2007), the same results concerning the late negativity with regards to rare stimuli were obtained during sleep

stage N2 and even SWS. Additionally, the authors reported that enhanced theta power was associated with the processing of rare stimuli, suggesting that theta power during sleep might be related to sensory/attentional processing of auditory stimuli. However, it is still a matter of debate whether these findings are really specific for sensory memory (A. M. Ibáñez, Martín, Hurtado, & López, 2009). Our results extend this interpretation by suggesting that large negativities after auditory stimuli presented during sleep might also support processes of long-term memory formation. In sum, our results demonstrate that cued reactivation of foreign words during sleep enhances vocabulary learning and that these processes are accompanied by distinct neuronal activities which involve sleep-specific slow oscillatory mechanism but possibly also share some properties with theta-related oscillations typically observed during successful encoding during wakefulness. Our findings suggest that verbal cueing of foreign vocabulary during post-learning sleep might be an efficient and effortless tool to improve foreign vocabulary learning in educational settings as well as every-day life.

Supplementary Information

Supplementary Table 1. Dutch words and German translations used in the learning task (English translations in brackets).

rundvlees	Rindfleisch (beef)	speeksel	Speichel (saliva)	buis	Rohr (pipe)
tijdschrift	Zeitung (newspaper)	veer	Feder (feather)	kerk	Kirche (church)
jukbeen	Jochbein (cheekbone)	vuur	Feuer (fire)	speelgoed	Spielzeug (toy)
soep	Suppe (soup)	bontjas	Pelzmantel (fur coat)	eiwit	Eiweiss (albumen)
hoogleraar	Professor (professor)	strook	Streifen (stripes)	vijg	Feige (fig)
spijs	Brei (mash)	mees	Meise (tit)	lat	Latte (batten)
draak	Drache (dragon)	veld	Feld (field)	moeras	Sumpf (bog)
maanlicht	Mondlicht (moonlight)	eetlepel	Esslöffel (tabelspoon)	brandstof	Brennstoff (fuel)
vacantie	Ferien (holiday)	penseel	Pinsel (brush)	veter	Schuhband (shoelace)
steen	Stein (stone)	sluis	Schleuse (watergate)	ijskast	Kühlschrank (fridge)
woordenboek	Wörterbuch	snuit	Schnauze (muzzle)	rits	Reihe (line)
naam	Name (name)	walm	Qualm (fume)	tegel	Fliese (tile)
werf	Hof (court)	beugel	Bügel (bow)	trui	Trikot (tights)
inkt	Tinte (ink)	duim	Daumen (thumb)	draad	Faden (wire)
zwaan	Schwan (swan)	munitie	Munition (munition)	ijzer	Eisen (iron)
griep	Grippe (flu)	bijl	Axt (axe)	stof	Staub (dust)
kraal	Koralle (coral)	boter	Butter (butter)	pols	Puls (pulse)
steiger	Gerüst (stage)	molen	Mühle (mill)	krijt	Kreide (chalk)
boom	Baum (tree)	onderdak	Unterkunft	vuil	Schmutz (dirt)
kogel	Kugel (bowl)	dal	Tief (low)	eierdooier	Eidotter (yolk)
bovenarm	Oberarm (upper arm)	poort	Tor (gate)	olijf	Olive (olive)
stoom	Dampf (steam)	werktuig	Werkzeug (tool)	servet	Serviette (napkin)
grond	Boden (ground)	wol	Wolle (wool)	been	Knochen (bone)
kuit	Laich (spawn)	halfrond	Halbkugel (hemisphere)	eikehout	Eichenholz (oak wood)
gangpad	Durchgang (passage)	schuur	Schuppen (hovel)	navel	Nabel (navel)
pot	Topf (pan)	kraag	Kragen (collar)	zitkamer	Wohnzimmer (living
borstkas	Brustkorb (chest)	lever	Leber (liver)	kermis	Jahrmarkt (carnival)
netvlies	Netzhaut (retina)	knoopsgat	Knopfloch (buttonhole)	ketting	Kette (chain)
goud	Gold (gold)	mol	Maulwurf (mole)	watten	Watte (cotton)
vorst	Frost (frost)	aroma	Geruch (smell)	amandel	Mandel (almond)
zakdoek	Taschentuch	dansvloer	Tanzfläche (dancefloor)	melk	Milch (milk)
regenbui	Regen (rain)	landschap	Landschaft (landscape)	zweep	Peitsche (whip)
gist	Hefe (barm)	twijg	Zweig (branch)	suikerriet	Zuckerrohr (sugar cane)
kruid	Gewürz (spice)	bagage	Gepäck (lugagge)	schrift	Heft (issue)
gijzelaar	Geisel (victim)	pamflet	Flugblatt (flier)	kleurstof	Farbstoff (stain)
gat	Loch (hole)	aarde	Erde (earth)	berk	Birke (birch)
herberg	Wirtshaus (tavern)	schouder	Schulter (shoulder)	stroop	Sirup (treacle)
zoogdier	Säugetier (mammal)	maaltijd	Essen (meal)	boord	Ufer (shore)
rubber	Gummi (gum)	snavel	Schnabel (beak)	folder	Prospekt (brochuer)
lunch	Mittagessen (lunch)	straat	Strasse (street)	lessenaar	Lesepult (lectern)

Supplementary Table 2. Behavioral data of "Gains" and "Losses" for cued and uncued words in each experimental group

		Cued	Uncued	*t*	*P*
Gains					
	Sleep	4.73 ± 0.65	2.93 ± 0.35	2.46	0.02*
	Sleep control	2.67 ± 0.57	2.53 ± 0.41	0.22	0.82
	Active waking	2.58 ± 0.37	2.23 ± 0.32	0.89	0.89
	Passive waking	2.94 ± 0.51	2.71 ± 0.31	0.41	0.68
Losses					
	Sleep	3.20 ± 0.52	4.80 ± 0.48	-2.53	0.02*
	Sleep control	4.67 ± 0.46	4.87 ± 0.40	-0.33	0.74
	Active waking	6.76 ± 0.83	6.05 ± 0.72	0.85	0.41
	Passive waking	9.35 ± 1.11	7.29 ± 0.67	1.83	0.08
Gains minus Losses					
	Sleep	1.53 ± 0.79	-1.86 ± 0.69	3.52	0.003**
	Sleep control	-2.00 ± 0.69	-2.33 ± 0.71	0.42	0.67
	Active waking	-4.17 ± 0.95	-3.82 ± 0.87	-0.37	0.71
	Passive waking	-6.41 ± 1.38	-4.58 ± 0.71	-1.26	0.22

Data are means ± s.e.m; "Gains": cued words, which were not remembered in the pre- but remembered in the post-sleep test. "Losses": cued words, which were remembered before sleep, but not after sleep. *: $P < 0.05$; ** $P < 0.01$.

Supplementary Table 3. Number of cueings specific to conditions and sleep stages

	Gains	Losses	HitHit	MissMiss	Control
Overall	52.1 ± 6.1	33.1 ± 5.8	272.1 ± 8.9	255.8 ± 10.1	302.2 ± 9.9
Percent (%)	5.8 ± 0.8	3.5 ± 0.6	29.7 ± 0.6	27.8 ± 0.6	32.9 ± 0.4
N2	21.5 ± 3.8	15.5 ± 2.9	126.0 ± 12.9	120.2 ± 11.8	142.8 ± 13.6
Percent (%)	5.5 ± 0.8	3.6 ± 0.6	29.3 ± 0.6	28.2 ± 0.8	33.3 ± 0.3
SWS	30.6 ± 5.7	17.6 ± 3.4	146.1 ± 17.5	135.6 ± 13.9	159.4 ± 18.5
Percent (%)	6.6 ± 1.1	3.5 ± 0.5	29.6 ± 0.8	28.1 ± 0.6	32.1 ± 0.1

Data are means ± s.e.m; "Gains": cued words, which were not remembered in the pre- but remembered in the post-sleep test. "Losses": cued words, which were remembered before sleep, but not after sleep. "HitHit": cued words, which were remembered before sleep and after sleep. "MissMiss": cued words, neither remembered before sleep nor after sleep. N2: NonREM sleep stages N2, SWS: slow-wave sleep. The upper line of each row indicates the absolute values, the lower line indicates the relative amount in percent

Supplementary Results:

Behavioral analysis of "Gains" and "Losses"

On the behavioral level, we analyzed "Gains" and "Losses" in all four experimental groups (main sleep group, sleep control group, active waking and passive waking). The analysis revealed that cueing during sleep increased the number of "Gains" and reduced the number of "Losses" in the sleep group, but not in the three control groups (3-way interaction between the factors "Gains vs. Losses", "cued vs. uncued" and "condition"; $F_{(3,60)} = 3.59$, $P = 0.019$). Post hoc tests indicated that only in the main sleep group, "Gains" as well as "Losses" significantly differed between cued and uncued words (both $P = 0.02$, see Table 3), resulting in significantly more "Gains" than "Losses" for cued as compared to uncued words in this group ($P = 0.003$). No effect of cueing was observed for "Gains" and

"Losses" in the sleep control group ($P > 0.29$) as well as in the two waking groups (all $P > 0.20$, except for a marginally increase in "Losses" by cueing in the passive waking group ($P = 0.08$), see Table 3). Please note that every cued word was repeated 10–11 times during the sleep period, resulting in sufficient number of trials for the EEG-analysis.

Relation of sleep stage specific cueing and reactivation advantage:

In order to disentangle more precisely a potential relationship between cueings in specific sleep stages (sleep stage N2 and SWS) and memory advantage of cueings, we correlated a "sleep stage cueing factor" (number of cueings per condition in SWS minus number of cueings per condition in sleep stage N2; conditions: "Gains", "Losses", "HitHit", "MissMiss") with the cueing success (memory for uncued words subtraced from cued words). None of these correlations reached significance (all $P > 0.35$).

Impact of pre-retention interval performance on cueing success

Since the vocabulary learning performance before the retention interval differed between participants (range 40–82 remembered words out of 120, respectively 33.3–68.3 %), we were interested if there would be a connection between this performance level and subsequent cueing success. In order to model cueing success, we subtracted memory for uncued words from cued words. Interestingly, and in line with a previous finding (Bergmann et al., 2012), we found in each of the three experimental cueing groups (main sleep group, active and passive waking group) an association between the pre-retention interval performance and the memory advantage induced by cueing (main sleep group: $r = 0.88$, $P < 0.001$; active waking: $r = 0.71$, $P = 0.001$; passive waking: $r = 0.697$, $P = 0.002$; the correlation coefficients did not differ significantly between groups (all $P > 0.2$); for

illustration see Supplementary Fig. 4). Thus the better participants were during learning, the stronger was the effect of cueing irrespective of sleep or waking. Further studies are needed to investigate this association systematically.

Sleep stage specific ERP analysis:

To examine sleep stage specific influences on event related potentials, we additionally analyzed ERPs for cuings during sleep stage N2 and SWS separately. The same time window (800 – 1.200ms) and electrode site (Fz) as in the main analysis was chosen. Please note that only subjects were included which had a minimum of 20 trials per condition in the particular sleep stage. Thus 10 subjects entered the analysis for sleep stage N2 and 12 subjects for SWS. Generally, results for ERP amplitudes were closely comparable between words presented during sleep stage N2 and SWS sleep. For example, ERP amplitudes for "Gains" were more negative than losses in sleep stage N2 (t_9= -2.21, P = 0.054, see Supplementary Fig 2 a - d) as well as in SWS (t_{11}= -2.95, P = 0.013, see Supplementary Fig 2 e - h). Importantly, we observed no significant interaction for the amplitude difference between "Gains" and "Losses" in sleep stage N2 vs. SWS ($F_{1,20}$ = 0.005, P = 0.94). Thus, our results concerning event related potentials differ just negligible between sleep stage N2 and SWS (Supplementary Fig.2), indicating that the results of our overall analysis, merging both sleep stages, represent a stable pattern of activity, with a more pronounced negativity associated with successful cueing.

Sleep stage specific induced theta power analysis:

As with the sleep stage specific analysis of event related potentials we additionally analyzed induced power changes for the theta band separately for sleep stage N2 and SWS.

The same time window (700 – 900ms) and electrode site (FC6) as in the main analysis was chosen. Results were generally similar between sleep stage N2 and SWS (see Supplementary Fig 3). However, the difference in theta power between "Gains" and "Losses" was stronger during sleep stage N2 (t_9= 4.60, P = 0.001) as compared to SWS (t_{11}= 2.44, P = 0.035) as indicated by a significant interaction between these two factors ($F_{1,20}$ = 4.39, P = 0.05). Furthermore, the increase in theta power mainly occurred at right frontal electrodes, while the topography during SWS included both right frontal and left parietal regions as reported in the main analysis including both sleep stages (see supplementary Fig. 3 c and f). Interestingly during SWS an additional increase in slow spindle activity associated with "Gains" (10 – 13 Hz) emerged between 600 and 800ms (electrode FC6 ($F_{4,44}$ = 2.67, P = 0.045), with significant stronger slow spindle power associated for "Gains" than for "Losses" (t_{11}= 2.44, P = 0.028); see Supplementary Fig. 3 g - i).

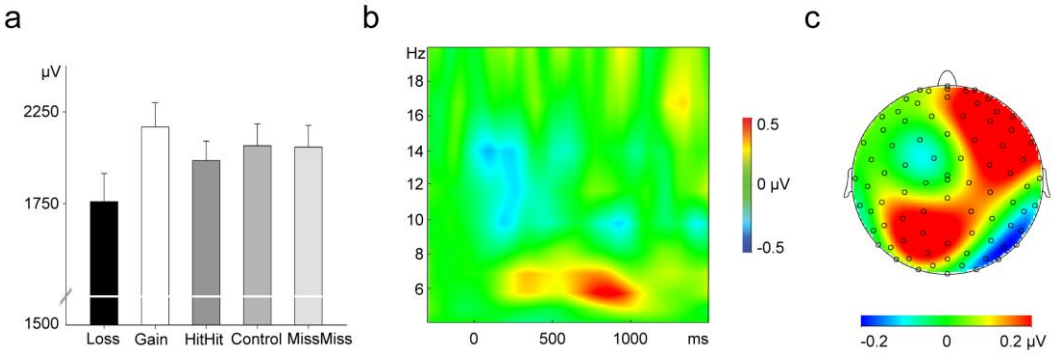
ERP and oscillatory theta activity during waking

As with the main sleep group we analyzed event related potentials and theta power with regards to three of the five different conditions ("HitHit", MissMiss" and "Control"). Due to the small trial number concerning "Gains" in both waking groups, we abstained from calculating differences between "Gains" and "Losses". With regards to ERP analysis we selected in accordance with previous findings concerning subsequent memory effects three different time segments for statistical analysis (i.e.: 0 – 300ms, 400 – 800ms and 100 – 1.200ms). Concerning the active waking group we could not find any significant difference in the three time segments between "HitHits" and "MissMiss" (all P = >0.4). In the passive waking group ERP amplitudes at electrode site Fz differed significantly in the early time window (0 – 300ms), with amplitudes associated with "HitHits" being more positive then

"MissMiss" (t_{16} = 2.18, P = 0.046). Concerning the middle time window (400 – 800ms) a statistical trend emerged (t_{16} = 2.07, P = 0.054), while the amplitude difference did not differ in the late time window (1.000 – 1.400ms; for illustration concerning the ERP results of both waking groups please see Supplementary Fig. 5).

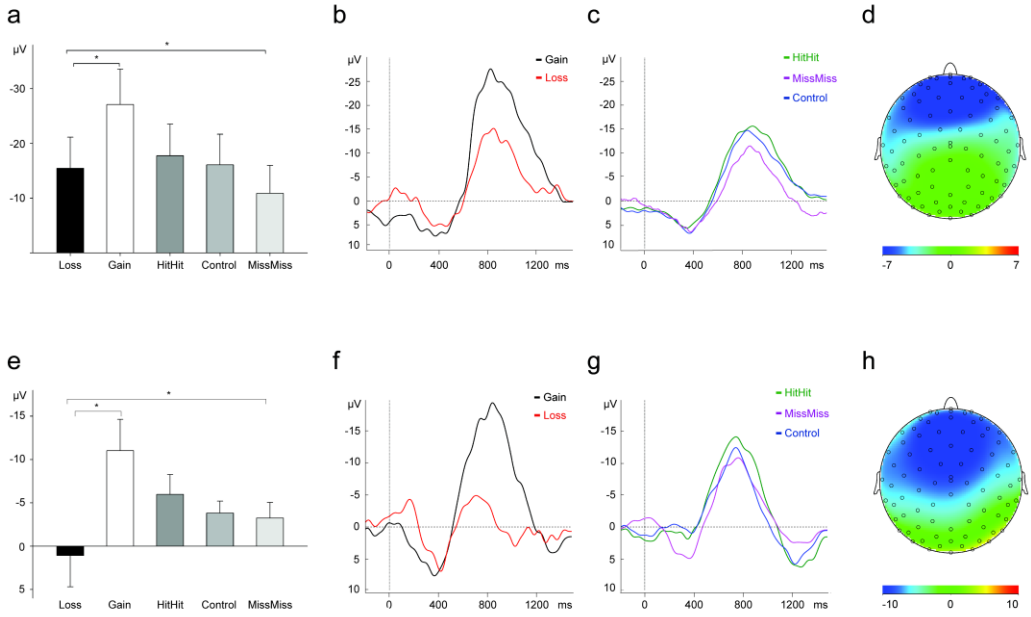
Similarly to the calculation of the EEG effects during sleep, a false discovery rate (FDR) of $P < 0.05$ was used to protect against error inflation due to multiple testing of multiple electrodes. None of the electrodes in the passive waking group survived the statistical threshold of $p < 0.05$ (FDR-corrected). Furthermore, we analyzed oscillatory theta activity in both waking groups with regards to "HitHits" and "MissMiss". We could not find any differences in theta power concerning those two conditions neither in the active nor in the passive waking group.

Supplementary Figure 1. Total theta power



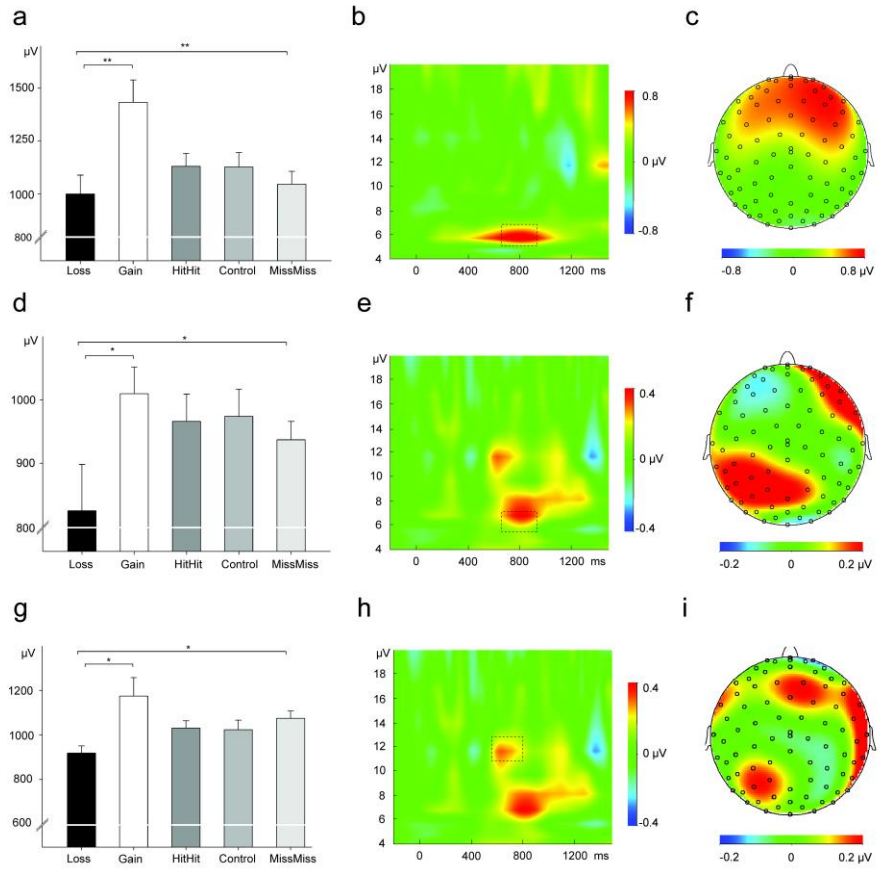
Total theta power for the different conditions at electrode FC6. (b) Difference in total power between "Gains" (c) Scalp map depicting the topographical distribution. As with the calculation of the induced theta power, a false discovery rate (FDR) of $P < 0.05$ was used to protect against error inflation due to multiple testing of multiple electrodes. None of the electrodes survived the statistical threshold of $p < 0.05$ (FDR-corrected).

Supplementary Figure 2. Sleep stage specific ERP analysis



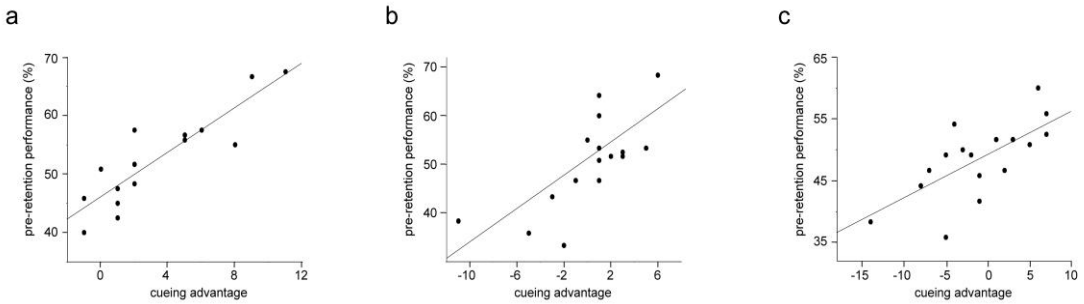
Event-related potentials (ERPs) associated with successful cueing during N2 sleep (a – d) and SWS (e – h): (**a**) Successful cueing during sleep stage 2 was associated with a more pronounced negativity at frontal electrode sites (representative electrode Fz). To simplify readability ERPs for Gains and Losses (**b**) respectively HitHits, MissMiss and the Control condition (**c**) are plotted separately. (**d**) Scalp map representing the topographical distribution for the difference between "Gains" and "Losses" in the time window between 800 and 1.100ms, indicating a pronounced frontal distribution. (**e - f**) The same pattern of event related activity with regards to the 5 different conditions emerged for words being presented during SWS.

Supplementary Figure 3. Sleep stage specific induced theta power analysis



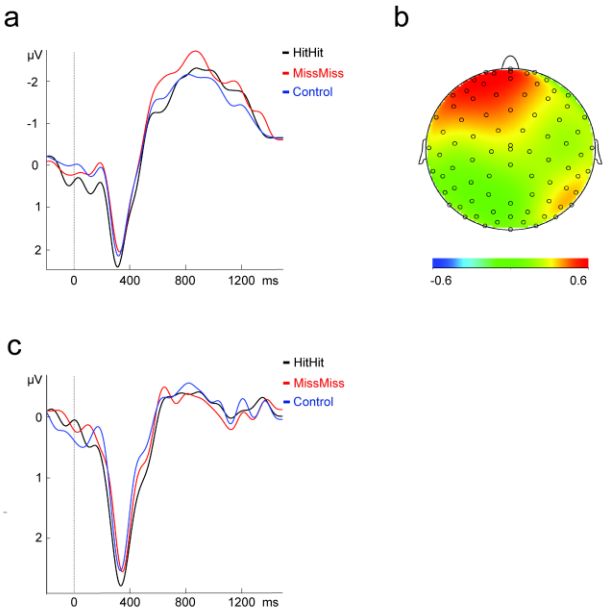
Oscillatory correlates of successful cueing during N2 sleep (a - c) and SWS (d – i). (**a + b**) Successful cueing during sleep stage 2 was associated with a more pronounced induced theta power (representative electrode FC6). (**c**) Scalp map representing the topographical distribution for the difference between "Gains" and "Losses" in the time window between 700 and 900 ms, indicating a pronounced frontal distribution. (**d + e**) Also during SWS successful cueing was associated with stronger induced theta power. (**f**) The topographical distribution differed from cueing during sleep stage 2 as the frontal effect was more focused on the right side and additionally a left parietal effect emerged. (**g + h**) Furthermore successful cueing during SWS was associated with enhanced oscillatory power in the range of slow spindles (11 – 13 Hz) succeeding the replayed words. (**i**) Scalp map representing the topographical distribution for the difference between "Gains" and "Losses" in the slow spindle range in the time window between 600 and 800 ms, indicating a right frontal and left parietal distribution.

Supplementary Figure 4. Pre-retention interval performance and cueing success



The pre-retention interval performance was correlated in each experimental cueing group with cueing success: (**a**) main sleep group (r = 0.88, $P < 0.001$); (**b**) active waking group (r = 0.71, $P = 0.001$); (**c**) passive waking group (r = 0.697, $P = 0.002$).

Supplementary Figure 5. ERP correlates of cueing during waking



ERP's to cued words during passive (a + b) and active waking (C): (**a**) ERP's at electrode site Fz for "HitHits", "MissMiss" and "Control" during passive waking. Amplitude differences between "HitHits" and "MissMiss" reached significance in a time window of 0 - 300ms and a statistical trend between 400 – 800 ms (**b**) Scalp map representing the topographical distribution for the difference between "HitsHits" and "MissMiss" in the time window between 400 and 800 ms. (**c**) ERP's at electrode site Fz for "HitHits", "MissMiss" and "Control" during active waking. Here the differences in amplitudes did not reach significance, therefore the topographical distribution is not shown.

Three

When less is more: Auditory feedback blocks memory benefits of cueing during sleep.

Thomas Schreiner
Björn Rasch

(submitted manuscript)

Abstract

Memories are spontaneously reactivated during sleep, and it is widely assumed that reactivation underlies the beneficial role of sleep on memory consolidation. Several studies have shown that re-exposure to memory cues during sleep reactivates memories and improves later recall. It is an intriguing question whether reactivated memories are sensitive for incorporating further auditory information after the cue, thereby strengthening the associative memory trace or possibly implanting false associations. We show that the benefits of cueing Dutch vocabulary during sleep are completely blocked when memory cues are followed by both correct and conflicting feedback. While successful reactivation is associated with an increase in oscillatory theta and spindle activity, presentation of auditory feedback unspecifically suppressed these patterns. Thus, memory cues are more efficient in stabilizing memories during sleep than cues followed by feedback, while oscillatory processes in the theta and spindle range are necessary for the memory benefits of reactivation during sleep.

Introduction

Reactivation plays a crucial role in the maintenance of long-term memories. Memory reactivation underlies almost all active recall processes, intentionally bringing the memorized information from memory to the focus of our attention(Bosch, Jehee, Fernández, & Doeller, 2014; Gisquet-Verrier & Riccio, 2012; Kuhl & Chun, 2014). Repeated active and successful recall attempts are known to be particularly effective in further strengthening of the memory trace for the long-term(Karpicke & Roediger, 2008) and might represent a core mechanisms for forming abstract and semantic long-term memories (i.e., multiple trace theory(Nadel, Samsonovich, Ryan, & Moscovitch, 2000)). Memories can also be reactivated by exposure to associated memory cues, thereby facilitating intended memory recall(Tulving & Thomson,

1973) or inducing unintentional memory retrieval(Mace, 2008). The consequence of reactivation by cueing on later memory strength might be twofold: If cued reactivation is immediately followed by interfering inputs, the reactivated memory might be destabilized and even forgotten(Nader & Hardt, 2009). In contrast, presentation of a cue followed by correct feedback (i.e. the associated item in paired-associated learning etc.) mostly reinforces the to-be-learned memory association and leads to a strengthening of the memory trace(Kilb & Naveh-Benjamin, 2011). Thus, the fate of a memory after its reactivation appears to strongly depend on the degree of overlap between the expected and the true input after reactivation. i.e. the resulting prediction error(Ben-Yakov & Dudai, 2011; Forcato, Fernandez, & Pedreira, 2014; Hardt, Einarsson, & Nader, 2010). In addition to waking, reactivation is also assumed to underlie the beneficial role of sleep for memory (Rasch & Born, 2013). According to the active system consolidation hypothesis, memories are spontaneously reactivated during Non rapid-eye movement (NonREM) sleep and thereby foster the strengthening and integration of the newly acquired memories in cortical knowledge networks(Diekelmann & Born, 2010; Genzel et al., 2014; Stickgold & Walker, 2013). Reactivation processes are assumed to be coordinated by cortical slow oscillations (< 1Hz). They are given the role of a time giving pace maker, orchestrating neural processes related to the reactivation of prior encoded memories. It is suggested that slow oscillations drive repeated reactivations of memory representations in the hippocampus together with sharp wave ripples. Since the slow oscillations simultaneously drive thalamo-cortical sleep spindles, they enable the formation of spindle ripple events, a mechanism that is assumed to enable the redistribution of reactivated hippocampal memory information to neocortical long term stores, leading to a subsequent stabilization and strengthening of the reactivated memory(Diekelmann & Born, 2010).

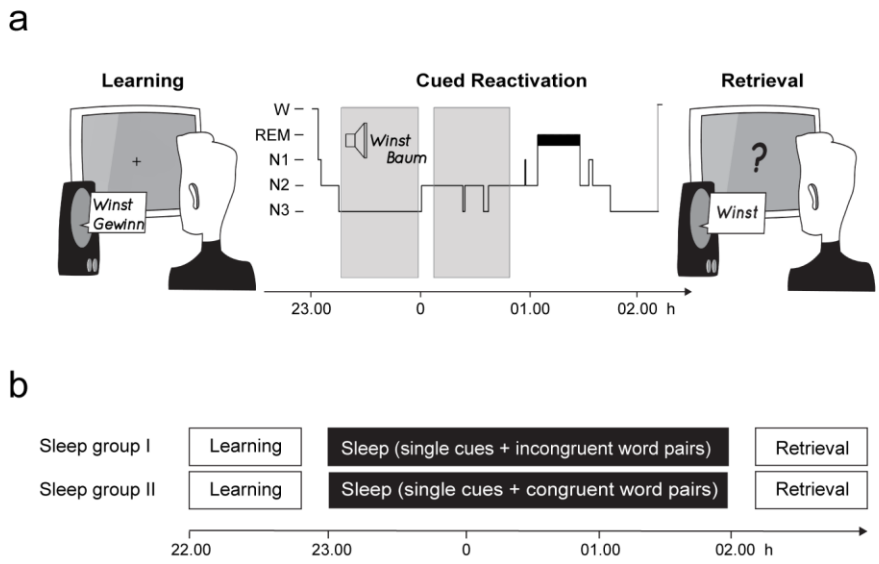
Various recent studies have established a causal role of reactivation for consolidation processes during sleep by showing that targeted reactivation during NREM sleep by cueing improves later memory recall(Antony et al., 2012; Rasch et al., 2007; Rihm et al., 2014; Rudoy et al., 2009). In these studies, memory cues (i.e., olfactory, auditory) are repeatedly presented during sleep to reactivate the associated memory content, leading to a strengthening of the memory tested after sleep. However the processes necessary for stabilizing memory representations after their reactivation during sleep are not well understood. In particular, it is still unknown whether additional input after cueing during sleep might improve or interfere with ongoing stabilization processes, and whether these effects are dependent on the degree of overlap between expected and true input as during wakefulness.

Here we aimed at identifying the critical processes supporting a strengthening of memories upon their reactivation during sleep. To test the role of a reactivation-associated prediction error during sleep, we presented correct vs. false feedback after reactivation by cueing during sleep and examined the underlying oscillatory correlates in healthy human subjects. Recently we could show that foreign vocabulary cues are capable of inducing reactivation processes and thereby boosting memory performance(Schreiner & Rasch, 2014). German-speaking participants had to learn Dutch German word pairs. During subsequent Non-REM sleep half of the Dutch words were replayed via loudspeaker leading to enhanced memory for the German translation of the cued words. We demonstrated that strengthening of memories by cueing during sleep was associated with a temporary increase in theta as well as spindle activity after re-exposure to verbal memory cues.

Here we basically used the same paradigm, but additional introduced auditory feedback (i.e., correct or false German translations) after cue presentation during NREM

sleep for some Dutch words ("cued words with feedback"). In the following we refer to the Dutch words as cues and the German words as feedback, which was correct or false with respect to the initially learned association between the Dutch word and the German translation. False feedback was created by randomly intermixing Dutch-German pairs from a subset of the original learning list. In total 27 healthy subjects participated in two experimental groups (correct feedback group: N = 14; false feedback group: N = 13). After the learning of 120 Dutch German word pairs, subjects slept for 3 hours in the sleep laboratory. During sleep stage N2 and slow wave sleep (SWS) a selection of the prior learned vocabulary was replayed. In the correct feedback group, Dutch cues replayed during sleep were immediately followed by the correct German translation. In the false feedback group, Dutch cues were followed by an incorrect German translation. In both groups, a third of the Dutch cues were always played without any feedback ("cued words"), and another third of the Dutch words was not played at all ("uncued words"). Following the sleep interval, memory for the German translations was tested. We hypothesized that the fate of a reactivated memory during sleep depends on the degree of prediction error after its reactivation: We expected that presentation of correct feedback after cueing leads to an additional improvement in later memory recall of the German translation whereas exposure to interfering, false feedback should result in increased forgetting of the Dutch vocabulary after sleep. In addition we attempted to extract neural activity associated with successful cueing using high-density EEG. We concentrated on oscillatory responses to vocabulary cues during sleep in the theta band, since theta activity was highly associated with cueing success in our recent study, and in the sleep spindle range, since previously we found as well hints that activity in the spindle range might be associated with cueing success and because of their assumed involvement in processes of sleep-dependent memory consolidation

Figure 1: Experimental procedure



(**a + b**) After studying 120 Dutch-German word pairs in the evening, participants slept for 3 hours. During the retention interval 80 Dutch words (40 cued, 40 cued + feedback) were repeatedly presented. The type of feedback for the "cued + feedback" words differed between the two experimental groups. While the correct feedback group received the correct German translation after the Dutch word during sleep, the false feedback group received an incorrect German translation. In the cueing categories, the proportion of remembered and forgotten word pairs of the last learning trial was maintained. Cueing of vocabulary occurred during NonREM sleep. After the retention interval, participants were tested on the German translation of the Dutch words using a cued recall procedure.

Results

Effects of verbal cueing on memory for Dutch vocabulary

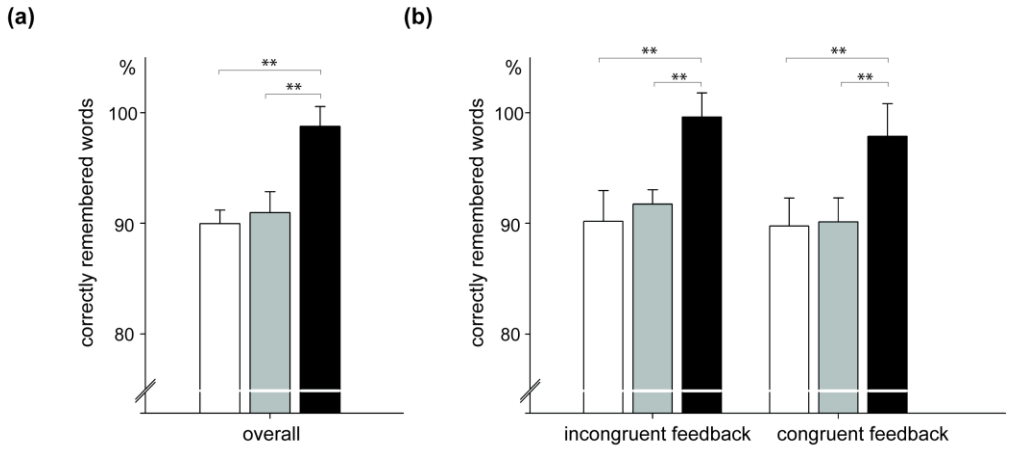
Consistent with our previous work(Schreiner & Rasch, 2014) re-exposure to Dutch cues during NREM sleep without additional feedback significantly improved memory recall after sleep in both experimental groups, by roughly 10% points as compared to "uncued" words (98.78 ± 1.78 vs. 89.98 ± 1.89%, $t_{26} = 4.61$, $P < 0.001$, see Fig. 2a). Astonishingly, providing auditory feedback after the Dutch cue completely blocked this beneficial effect of cueing during sleep: Participants remembered only 90.98 ± 1.22 word pairs of the "cued +

feedback" category, which was significantly lower than memory for "cued" words without feedback (t_{26} = 4.71 , P < 0.001). Performance level for "cued + feedback" words was basically identical as compared to uncued words (t_{26} = 0.52, P > 0.60, see Table 1 for descriptive values).

Most importantly, the type of feedback was completely irrelevant for this effect: Correct as well as false feedback after cueing during NREM sleep similarly blocked memory benefits both in the correct feedback group (90.14 ± 2.17%) as well as in the false feedback group (91.75 ± 1.29%, see Fig. 2b, for pairwise comparisons). Memory performance in the "cued + feedback" category did not differ between the experimental groups (P > 0.50). The results pattern was substantiated by a significant overall main effect of cueing category ("cued + feedback", "cued" and "uncued", $F_{(2,50)}$ = 13.34, P < 0.001) and no interaction with the factor "type of feedback" (correct vs. false, $F_{(2,50)}$ = 0.76, P >0.90).

Thus, we could successfully replicate our previously reported findings that re-exposure to vocabulary cues during sleep without feedback improves memory for the German translations as compared to uncued words(Schreiner & Rasch, 2014). In contrast to the benefits of vocabulary cueing during sleep, re-exposure to cues + feedback during sleep blocks the benefits of cueing, irrespective of whether the feedback is correct of false. Neither did the re-exposure to false feedback further deteriorate subsequent memory performance or implanted false associations, nor did the replay of correct feedback additionally improve later memory. The results pattern suggests that the mere presentation of a second auditory stimulus after cueing disrupts underlying neural processes critical for memory benefits of cueing during NREM sleep.

Figure 2: Behavioral results



(a) Overall, memory for "cued" words (black bar) was significantly improved as compared to "uncued" (white bar) and "cued +feedback" words (grey bar). **(b)** The same pattern of results is visible in both sleep groups. In the false sleep groups as well as the correct sleep group memory for "cued" words (black bar) was enhanced as compared to "uncued" (white bar) and "cued +feedback" words (grey bar). Retrieval performance is indicated as percentage of recalled German translations with performance before sleep set to 100%. Values are mean ± s.e.m. $^{**}P \leq 0.01$.

Table 1: Overview of memory performance

		Cue	Cue + Feedback	Uncued	*F*	*P*
False feedback group						
Cued recall	*Learning*	20.07 ± 1.08	20.50 ± 1.08	20.28 ± 1.02	2,07	0.14
	Retrieval	19.92 ± 1.04	18.79 ± 1.04	18.28 ± 1.09	5.21	0.01*
	Change	-0.15 ± 0.40	-1.71 ± 0.29	-2.00 ± 0.61	7.91	0.002**
	% Change	99.61 ± 2.19	91.75 ± 1.29	90.18 ± 2.80	8.04	0.002**
Correct feedback group						
Cued recall	*Learning*	20.61 ± 1.98	21.23 ± 1.95	21.07 ± 1.98	2.38	0.12
	Retrieval	20.23 ± 2.14	19.23 ± 1.93	19.23 ± 2.10	4.09	0.02*
	Change	-0.38 ± 0.54	-2.00 ± 0.46	-1.84 ± 0.44	7.44	0.003**
	% Change	97.88 ± 2.94	90.14 ± 2.17	89.76 ± 2.54	5.74	0.008**

Data are means ± s.e.m; Numbers indicate absolute or relative values of correctly recalled or recognized words that where presented during the retention interval ($^1/_3$ as cues, $^1/_3$ as cues + feedback; 80 in total) or not ($^1/_3$ uncued words, 40 in total). Please note that the false and the correct sleep group differed with regards to the feedback. While for flase sleep group incorrect feedback was replayed during sleep, feedback in the correct sleep group was correct. For cued recall testing, number of correctly recalled words during the learning phase before and the retrieval phase after the retention interval are indicated. Change (% Change) refers to the absolute (relative) difference in performance between learning and retrieval phases. $^*P < 0.05$; $^{**}P < 0.01$.

Sleep and cueing

Online monitoring as well as offline analysis of the sleep EEG assured that presentation of auditory cues occurred during NREM sleep and did not lead to increased awakening responses (see Table 2, for sleep data). Sleep architecture did not significantly differ between the correct and the false feedback group (all $p > 0.23$). We excluded the occurrence of brief awakenings by showing that occipital alpha power 1000ms before and after each auditory cue did not differ (mean alpha power at electrode Oz: 1.37 ± 0.38 μV vs. 1.34 ± 0.28 μV; $t_{26} = 0.01$, $P = 0.92$). To investigate whether the success of verbal cues was related to time spent in a certain sleep stage, we computed a memory advantage score (i.e., by subtracting memory for cued minus uncued words (Antony et al., 2012)) and correlated this score with the relative time spent in each sleep stage for all participants. We did not observe any significant associations (N2: $r = 0.27$, $P = 0.16$; SWS: $r = -0.34$, $P = 0.08$; REM: $r = -0.13$, $P = 0.51$).

Table 2: Sleep parameter

	False feedback group	Correct feedback group	*P*
Duration [min]			
N 1	9.21 ± 1.74	11.46 ± 2.36	0.44
N 2	97.42 ± 4.81	102.03 ± 3.85	0.46
SWS	62.78 ± 4.18	58.34 ± 3.46	0.39
REM	20.85 ± 2.76	24.69 ± 2.73	0.23
WASO	1.35 ± 0.47	2.37 ± 1.05	0.77
Duration [%]			
N 1	4.65 ± 0.77	5.51 ± 1.01	0.51
N 2	50.70 ± 1.99	51.01 ± 1.84	0.81
SWS	32.97 ± 2.29	29.76 ± 1.81	0.22
REM	10.41 ± 1.42	12.29 ± 1.09	0.31
WASO	2.48 ± 0.68	2.26 ± 1.05	0.75

Data are means ± s.e.m. N1, N2: NonREM sleep stages N1 & N2, SWS: slow-wave sleep / N3, REM: rapid eye movement sleep, WASO: wake after sleep onset.

Neural correlates of cueing during sleep

To assess the blockade of cueing benefits on a neural basis, we analyzed oscillatory responses to vocabulary cues during sleep. Data of the correct and incorrect feedback group were collapsed, as the behavioral effects of cueing and feedback was identical in both groups. We controlled for a possible contribution of the evoked brain response by subtracting the evoked power from each single trial before calculating the time-frequency analysis (induced power)(Klimesch et al., 1998). Based on our previously reported analysis and results(Schreiner & Rasch, 2014), we analyzed oscillatory activity in the theta band in a time window of 500ms – 800ms after stimulus onset. Consistent with our previous findings, cued words without feedback that were correctly remembered after sleep were associated with an increased theta-oscillation (~6 Hz) as compared to subsequently forgotten cued words (t_{19} = 2.54, P = 0.02). The effect was most pronounced when comparing cued words that were correctly recalled after but not before sleep ("cued gains": 545.91,98 ± 19.54 μV) with those which had been known before but not after sleep ("cued losses" 466.46 ± 19.44 μV, t_{19} = 4.12, P = 0.001; for details see Fig. 3). The categories "gains" and "losses" reflect a clear behavioral change after cueing, therefore best representing the neural pattern associated with processes underlying successful vs. unsuccessful cueing for later memory retrieval. The increase in induced theta power between "cued gains" and "cued losses" had a stable fronto - central distribution (see Fig. 3). To further investigate the exact time course of the effect we compared theta activity for "gains" and "losses" in 100ms steps, ranging from 0 to 2500ms. The results indicated, after correcting for multiple comparisons, that theta activity associated with "gains" differed from "losses" in an early time-window from 400ms to 900ms and in a late time window ranging from 1800-2500ms (for details see Supplementary Table 3).

In sharp contrast to "cued" words, no significant effect associated to theta power emerged for "cued + feedback" words presented during sleep. Theta activity between 500-800ms neither differed between subsequently remembered vs. forgotten "cued + feedback" words ($P = 0.16$) nor between "cued + feedback gains" vs. cued + feedback losses" ($P = 0.74$). Additionally no difference for "gains" and "losses" was visible when comparing theta activity in 100ms steps (all $P > 0.3$). A direct comparison revealed, that theta power associated with Dutch words without feedback ("cued gains") was significantly stronger than theta power for Dutch words presented with feedback ("cued + feedback gains", $t_{19} = 2.19$, $P = 0.041$; time window: 500-800ms). This striking feedback-dependent difference in theta-effects which mirrored the behavioral result pattern was substantiated in an overall ANOVA by a significant interaction between the factors feedback ("cued" vs. "cued + feedback") and memory consequence ("gains" vs. "loss", $F_{(1,19)} = 9.31$, $P = 0.007$; for descriptives see Supplementary Table 2).

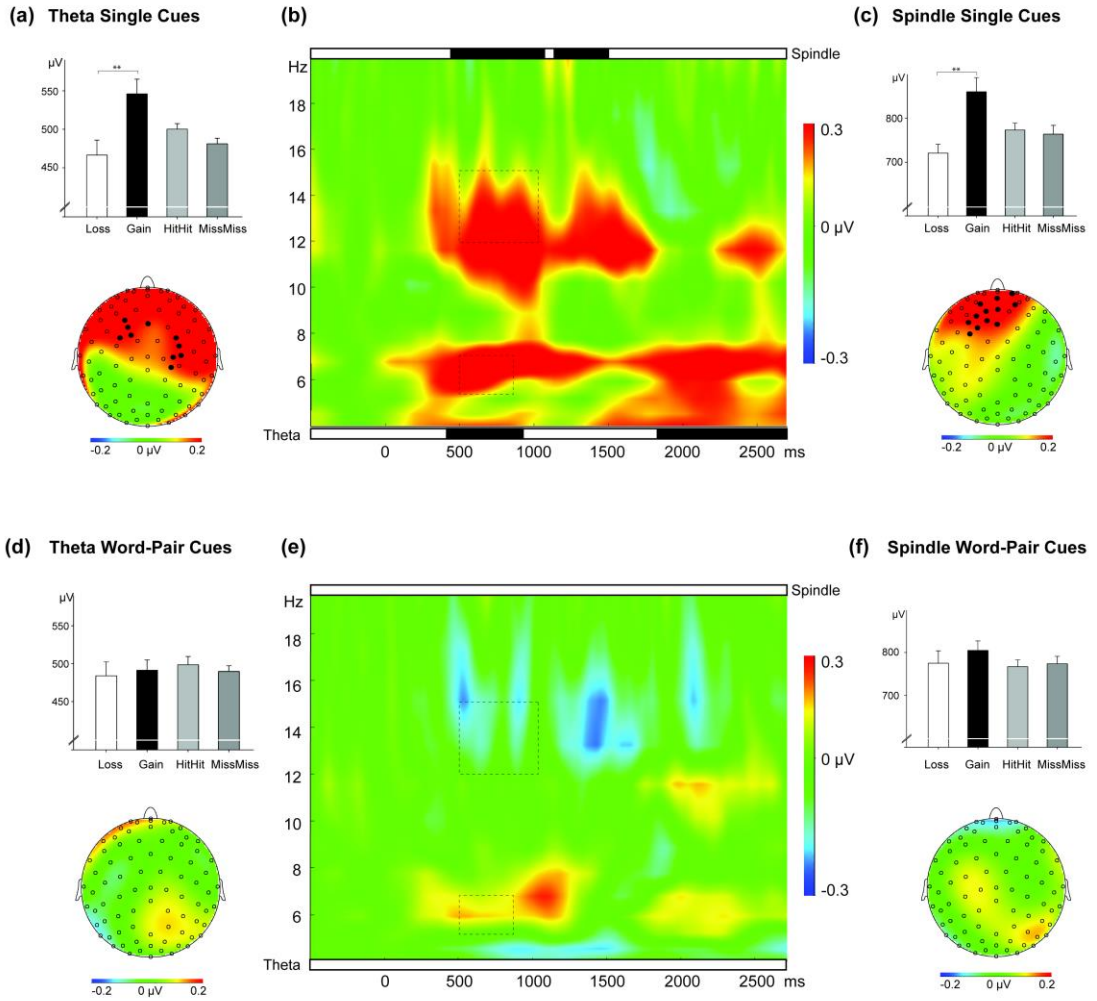
We further explored possible oscillatory differences with regards to our conditions in the spindle frequency range (~13 Hz). Similar to theta-power, we observed a significant increase in spindle power in the time window 500 – 1000ms after stimulus onset for successfully remembered "cued" words as compared to later forgotten words ($t_{19} = 2.39$, $P = 0.027$). The effect was most pronounced for "cued gains" vs. "cued losses" ($t_{19} = 3.57$, $P = 0.002$) and had a stable fronto - central distribution (see Fig. 3). Again we contrasted the temporal effects of "gains" and "losses" in 100ms steps. "Gains" differed from "losses" in a broad time window from 400 to 1500ms (for details see Supplementary Table 3).

Once more no significant effect associated to power in the spindle range emerged for "cued + feedback" words presented during sleep. Spindle power between 500-800ms

neither differed between subsequently remembered vs. forgotten "cued + feedback" words ($P = 0.23$), nor between "cued + feedback gains" vs. cued + feedback losses" ($P = 0.16$). No difference between "gains" and "losses" was visible when comparing theta activity in 100ms steps. Spindle power for gained "cued" words without feedback was higher as compared to gained "cued + feedback" words ($t_{19} = 2.16$, $P = 0.04$) and the interaction between the factors feedback ("cued" vs. "cued + feedback") and memory consequence ("gains" vs. "loss") was highly significant ($F_{(1,19)} = 15.31$, $P = 0.001$).

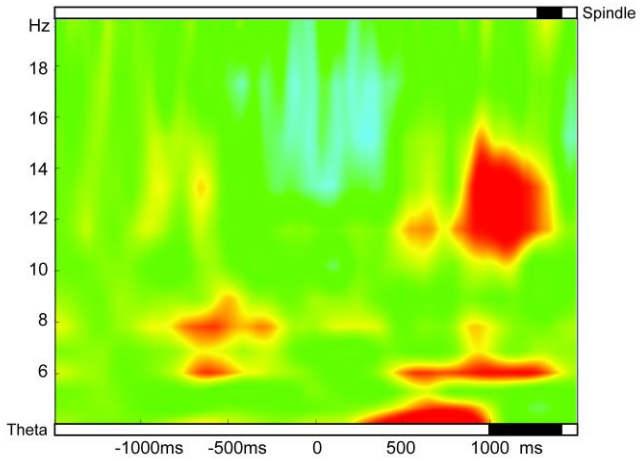
If the presentation of feedback indeed blocked the neural correlates of successful memory cueing during sleep, it might be possible that the feedback itself acted as a memory cue. During learning, participants first heard the Dutch word and then recalled the German translation. In spite of this clear direction in the associative learning procedure, it might still be possible that the German translation also weakly reactivated the associated Dutch word during sleep. As false feedback was created by randomly intermixing Dutch-German pairs taken from original learning list, here we recoded the categories of "gains" and "losses" in the false feedback group to adequately reflect behavioral gains and losses with respect to the feedback (and not the first word, as in the original analysis). Astonishingly after recoding, the behavioral "gains" with respect to the feedback were again associated with enhanced theta power in a late time window ranging from 1000ms to 1400ms (all $P<0.03$). Spindle activity differed as well in a short, late time frame from 1300 to 1400ms ($t_{19} = 2.25$; $P = 0.036$; see Fig. 4). The re-occurrence of increased theta and spindle activity associated with behavioral "gains" after the feedback presentation again suggests that oscillatory processes might be important for successful memory cueing during sleep and should not be disturbed by further auditory input.

Figure 3: Oscillatory results



Oscillatory theta and spindle power, recorded during cueing in both sleep groups, were computed for words, for which cueing during sleep led to a change in memory performance. "gains" reflect cued words not remembered in the pre-sleep test but correctly recalled in the post-sleep test. "losses" refer to cued words remembered in the pre-sleep test but not in the post-sleep test. Words remembered before and after the retention interval were labeled "hithit" and words not remembered both before and after the retention interval were labeled "missmiss". Successful cueing of single words was associated with enhanced power in the theta **(a)** and spindle **(c)** band. **(b)** Representative electrode F3. Verbal cues were presented at time 0 ms. The rectangle illustrates the time window used in the bar chart. Top and bottom panels indicate significant differences (in black) between "gains" and "losses" in spindle and theta power, respectively. **(d - f)** The differences in theta (d) and spindle band (f) vanished when word pairs instead of single cues were replayed during sleep.

Figure 4: Oscillatory results for second word cues



With regards to the feedback cues presented during sleep, theta and spindle power differed in a late time window (representative electrode Fz). The zero point refers to the onset of the German feedback cue. Top and bottom panels indicate significant differences (in black) between "gains" and "losses".

Cueing and slow oscillations

In a single trial analysis, we counted the number of clearly identifiable slow waves that followed cueing of "cued gains" words as compared to "cued losses", as well as the slow waves following the cues and the feedback, respectively of our "cued + feedback gains" as compared to "cued + feedback losses" categories. "Gains" were generally followed by an increased number of slow oscillations ($F_{(2,38)}$ = 36.33, $P > 0.001$, main factor "gain/loss"), while "cued gains" were associated with the highest number of slow oscillations, following the word presentation (($F_{(2,38)}$ = 9.56, $P > 0.001$, main factor "gain/loss"). The cues witch were followed by feedback presentations were associated with the smallest number of slow oscillations (cued gains from "cued + feedback" vs. "cued gains": t_{19} = 3.41; P = 0.003; "cued gains" vs. "feedback gains" with regards to "cued + feedback": t_{19} = -2.41; P = 0.049),

indicating that the presentation of feedback might have partly suppressed the emergence of slow oscillations (for descriptive data see Table 3).

Table 3: Number of slow oscillations

	Cue	Cue from "cued + feedback"	Feedback from "cued + feedback"	*F*	*P*
Gains	45.16 ± 3.02	33.06 ± 2.18	39.84 ± 2.16	6.39	0.005**
Losses	32.85 ± 3.21	24.53 ± 2.19	27.84 ± 81	3.61	0.034*

Data are means ± s.e.m; relative numbers of slow oscillations following word onset of cues and separately for the cues and the feedback of the "cue + feedback" category. *$P < 0.05$; ** $P < 0.01$.

Discussion

The present study contrasted for the first time the effects of presenting verbal cues vs. cues followed by correct and false feedback after cueing during sleep on later memory performance. The replay of foreign vocabulary cues improved later memory performance. In sharp contrast, presenting auditory feedback (i.e. German words) after the Dutch cue during sleep completely blocked the beneficial effect of cueing during sleep on later recall, independent of whether the feedback (i.e., the German translation) was correct or false. Furthermore, successful verbal cueing during sleep was associated with increased theta and spindle activity during Non-REM sleep. This neural pattern associated with cueing success vanished when verbal cues were followed by correct and false feedback, suggesting auditory feedback after cueing disrupts neural and oscillatory processes critical for memory stabilization after reactivation during sleep.

The enhancing effect of replaying vocabulary cues during Non-REM sleep on later memory performance, replicates a recent finding, were we could show that foreign vocabulary are capable of inducing memory reactivations during sleep(Schreiner & Rasch, 2014). The replication demonstrates the reliability of the beneficial effect of verbal cueing during sleep and adds further support to the assumptions of the active system consolidation theory, which postulates that spontaneous memory reactivations during sleep are essential for the beneficial effect of sleep on memory consolidation. Various studies have successfully used memory-associated cues such as odors or sounds to specifically strengthen declarative memories during sleep (Diekelmann et al., 2011; Rasch et al., 2007; Rudoy et al., 2009). In all these studies, memory cues were presented during sleep, which acted as a "reminder" to unintentionally reactivate an associated memory representation. However none of these studies presented the complete memory presentation or competing information during sleep.

Here we wanted to specifically test whether the beneficial effect of cueing during sleep would be altered if memory cues were followed by correct or false information, i.e. whether the degree of overlap between expected and true input after memory reactivation (i.e., the prediction error) is critical for subsequent stabilization processes during sleep. Our results show that presentation of auditory information after cueing completely blocks any beneficial effects of cueing, independent of the type of feedback. Our behavioral result pattern has at least two important implications: First, presentation of the whole memory presentation (i.e. cue + correct feedback") does not further strengthen memories during sleep. This result suggests that presentation of memory cues and the induction of unintentional memory reactivation appear to be necessary for the beneficial effects of cueing during sleep.

Second, presentation of interfering information after cueing during sleep (cue + false feedback) does not further impair memory retention or induce "false" memories intrusions. While there are some reports showing that learning of new association in simple conditioning paradigms might be possible during sleep(Arzi et al., 2012), our results provides no further support for the notion that presentation of interfering information after cueing induces forgetting, due to newly learned associations.

The fact, that both correct and false feedback similarly blocked the benefits of cueing during sleep favors an alternative explanation: Based on our behavioral results pattern, it seems highly probable that the presentation of a second stimulus might have unspecifically blocked or disturbed neural processes elicited through the first cue which are critical for the stabilization of reactivated memories during sleep. Thus, for verbal cueings reactivation-associated processes were allowed to proceed uninterrupted, thereby exerting its beneficial effects on later memory performance. The mere presentation of a second auditory stimulus, irrespective of its content, might have interfered with these processes, resulting in an absence of any behavioral effect. The interfering effect might have occurred on different levels: Additional auditory input after cueing might have disturbed ongoing reactivation processes, which are reported to persist until the next cue(Bendor & Wilson, 2012b) and also occur at a ca. 10 times faster time scale as external information processing(O'Neill et al., 2010). Furthermore, unspecific auditory processing after cueing might have interfered with ongoing oscillations critical for stabilizing the reactivated memory.

The time frequency results appear to favor the second alternative. Successful cueing during sleep (i.e. cueings leading to improved memory performance) was accompanied by a post-stimulus increase in induced theta and spindle power. These effects almost completely vanished when a second stimulus was replayed. Cueing associated neural processes were

visible starting approximately 500ms after word onset. Our cueing procedure was such that in case of cue + feedback, the feedback succeeded the cue at 200ms. Depending on the length of the cue (varying between 450 -700ms), presentation time of feedback fell as a consequence into a time range between 650 and 900ms. According to our cueing results, presentation of the feedback might have fallen into a critical time window, thereby blocking cueing related stabilization processes associated with oscillations in the theta and spindle range. Interestingly, slight increases in theta and spindle activity re-emerged after presentation of the feedback, suggesting that the feedback might have acted as memory cue to reactivate the associated memory representation in a reversed order, although without any clear behavioral consequences.

It is assumed that the co-occurrence of memory reactivations with spindle-ripple events synchronized by slow oscillations is critical for stabilizing the reactivated memory trace(Bergmann et al., 2012; Dongen, Thielen, et al., 2012; Rasch & Born, 2013; Rihm et al., 2014; Ritter et al., 2012). We did not synchronize the verbal cues to the phase of the slow oscillation, however, successful reactivated memories were more often followed by slow oscillations. Whether the occurrence of post-stimulus slow oscillations simply predicted cueing success or whether successful cueing resulted in an increase in slow oscillations remains to be determined. The finding that successful cueing of Dutch words during sleep is related to post-stimulus increases in oscillatory sleep spindle power is also in line with their assumed involvement in processes of sleep-dependent memory consolidation(Fogel & Smith, 2011). The relationship of sleep spindles and declarative memory processes has been demonstrated in various studies. Sleep spindles have been consistently linked to the amount of overnight retention of declarative memories, at least in women(Ujma et al., 2014). Specifically spindles have been associated with the learning of word pairs(Gais, Mölle,

Helms, & Born, 2002b; M Schabus et al., 2004), the integration of newly learned information into existing knowledge networks(Tamminen et al., 2010) and reactivation processes in humans(Bergmann et al., 2012), as well as in rodents(Johnson, Euston, Tatsuno, & McNaughton, 2010; Peyrache et al., 2009). Hence, numerous findings generally implicate sleep spindles in memory consolidation processes during sleep, which fits to our results and conclusions of a stabilizing role of oscillations in the spindle range on reactivated memory representations.

Additionally we found that successful cueing of Dutch words is accompanied by enhanced theta activity. As with spindle activity, successful cueing of Dutch words was associated with enhanced theta power, while no such difference was observable for cues followed by auditory feedback. The presentation of auditory feedback after the Dutch cue as well interrupted theta activity, which in turn blocked any behavioral effect of cueing during sleep. The presentation of feedback was also associated with enhanced theta and spindle power, in case that the matching Dutch-German word pair was gained through the cueing procedure during sleep. It has to be noted that the oscillatory effects linked to the feedback presentation were not as stable and strong as for word cues, which nicely mirrors the behavioral effects.

The result that successful cueing during sleep is associated with enhanced theta power replicates recent findings in adults(Schreiner & Rasch, 2014) as well as children (Wilhelm et al., submitted manuscript). In addition, faster theta frequency or increased theta power during NonREM sleep predicted better subsequent memory performance in patients with Alzheimer's disease or amnestic mild cognitive impairment and in healthy subjects(Hot et al., 2011; Manuel Schabus et al., 2005; Westerberg et al., 2012). A recent study demonstrated that spike timing during delta-nested theta rhythms controls a reciprocal

interaction between deep and superficial cortical layers mimicking the alternating cortical dynamics of sensory and memory processing during wakefulness(Carracedo et al., 2013). Despite this growing evidence connecting theta activity during sleep and processes of memory consolidation and reactivation, theta oscillations have not yet been included in theoretical accounts of sleep and memory. During wake, induced theta has been consistently linked to the successful encoding and retrieval of new declarative information(Klimesch, 1999; Nyhus & Curran, 2010b). Potential generators of this memory related theta activity have been reported in the cortex and the hippocampus(Lega, Jacobs, & Kahana, 2012; Sederberg, Kahana, Howard, Donner, & Madsen, 2003). In rodents, long term potentiation, which is thought to underlie learning and memory, can be induced in cortical areas as well as the hippocampus by stimulation trains that mimic the theta rhythm(Morgan & Teyler, 2001; Rioult-Pedotti, Friedman, & Donoghue, 2000; Werk & Chapman, 2003). Thus a causal role of theta oscillations during wake for synaptic plasticity is widely accepted. Our current findings together with recent studies suggests that theta oscillations may also play a crucial role for stabilizing reactivated memories during sleep. Further examination is needed to precisely determine the contribution of theta oscillations for stabilizing and strengthening memories during sleep.

Our results demonstrate that cued reactivation of foreign words during sleep enhances vocabulary learning, while the presentation cues + feedback, irrespective of content, blocked any behavioral effect. The beneficial effects of cueing were accompanied by enhanced oscillatory activity in the spindle and theta range, while presenting feedback vanished these effects, potentially disturbing neural and oscillatory processes critical for stabilizing and strengthening memories during sleep upon their reactivation.

Online Methods

Subjects

30 healthy, right-handed subjects (19 female, mean age = 22.00 ± 2.6) with German mother tongue and without Dutch language skills participated in the study. 3 subjects had to be excluded due to insufficient sleep. In the final sample, 14 volunteers participated in the correct feedback group (10 female, mean age = 22.7 ± 3.09 years) and 13 subjects in the false feedback group (9 female, mean age = 21.15 ± 1.95 years). Age and gender distribution did not differ between the experimental groups (both $P > 0.75$).

None of the participants were taking any medication at the time of the experiment and none had a history of any neurological or psychiatric disorders. All subjects reported a normal sleep-wake cycle and none had been on a night shift for at least 8 weeks before the experiment. On experimental days, subjects were instructed to get up at 7.00h and were not allowed to take in caffeine and alcohol or to nap during daytime.

The study was approved by the ethics committee of the Department of Psychology, University of Zurich. All subjects gave written informed consent prior to participating. After completing the whole experiment participants were paid 120 Swiss francs.

Design and Procedure

Participants of both sleep groups entered the laboratory at 21.00h. The session started with the application of the electrodes for standard polysomnography, including electroencephalographic (128 channels, Electrical Geodesic Inc.), electromyographic (EMG) and electrocardiographic (ECG) recordings. Prior to the experiment, participants spent an adaptation night in the sleep laboratory.

The learning phase started at approximately 22.00h with the vocabulary-learning task (Dutch-German word pairs, for a detailed description see section Vocabulary Learning Task). After completing the learning task, participants of both sleep groups went to bed at ~ 23.00h and were allowed to sleep for 3 hours. During the 3-hour retention interval, a selection of the prior learned Dutch words was presented again during sleep stages N2 and N3 (SWS) for a total duration of 90 min (see below for a detailed description of the reactivation phase). At ~ 2.00h subjects of both sleep groups were awakened from sleep stage 1 or 2 and at ~ 2.15h, recall of the vocabulary was tested.

Vocabulary Learning Task

The vocabulary-learning task consisted of 120 Dutch words and their German translations, randomly presented in three learning rounds (word pairs are listed in the Supplementary Table 1). Dutch words and their german translations were presented aurally (duration range 450-700ms) via loudspeakers (70 dB sound pressure level). In the first learning round, each Dutch word was followed by a fixation cross (200ms) and subsequently by its German translation. The inter-trial interval between consecutive word pairs was 1,000-3,000ms. The subjects were instructed to memorize as many word pairs as possible. In a second round the Dutch words were presented again followed by a question mark (ranging up to 7 seconds in duration). The participants were instructed to vocalize the correct German word or to say, "next" (German translation: "weiter"). Afterwards, the correct German translation was presented again, irrespective of the correctness of the given answer. In the third learning round, the cued recall procedure was repeated without any feedback of the correct German translation. Recall performance of the third round (without feedback) was taken as pre-retention learning performance. In the third round, participants recalled on

average 61.55 ± 16.77 words (range 27 – 104 words) of the 120 words correctly, indicating an ideal medium task difficulty (recall performance 51.29%) without any danger of ceiling or floor effects. We observed no difference in pre-retention memory performance between the two experimental groups (t_{25} = -0.40; P = 0.69; see Table 1 for descriptive statistics).

Reactivation of vocabulary

In the reactivation phase during the 3-h retention interval the 120 Dutch-German word pairs were assigned to one of three categories each consisting of 40 stimuli: "cued + feedback", "cued" and "uncued". In the "cued + feedback" category, Dutch words were aurally presented repeatedly during NREM sleep directly followed by a German word. In the "cued" category, only Dutch words alone without any feedback were played, whereas uncued words were not presented during sleep. In all three categories, the proportion of remembered and forgotten word pairs of the last learning trial was maintained. All words were individually and randomly chosen for each participant using an automatic MATLAB algorithm.

While the categories "cued" and "uncued" were identical for all participants, the type of feedback for the "cued + feedback" words differed between the two experimental groups. The conrrect feedback group received the correct German translation after the Dutch word during sleep. In contrast, the false feedback group received an incorrect German translation. The false feedback was created by randomly intermixing the Dutch and German words of this category, thus new Dutch-German word combinations for replay during sleep were formed.

In total, 80 Dutch words (40 "cued + feedback" and 40 "cued" words) from the total of 120 Dutch-German word pairs were presented during NREM sleep via loudspeaker

(50 dB sound pressure level). Each replay trial during sleep started with the presentation of a Dutch word. For "cued + feedback" trials, the Dutch word was followed after a 200ms break by the respective German word. Thus each German word started 650 to 900ms after the respective Dutch word onset. In the case of "cued" Dutch words, the second stimulus was replaced by a silent audio file (0 dB, duration: 600ms). The inter-trial interval was 2.800-3.200ms. Replay occurred in a randomized order for a total of 90 minutes, resulting in ~17 exposures to each word (see Table 2). The rational of repeated cueing during sleep was derived from previous studies using olfactory or vocabulary cues which were repeated several times successfully induces memory reactivation during sleep(Diekelmann et al., 2011; Rasch et al., 2007; Rihm, Diekelmann, & Born, 2013; Schreiner & Rasch, 2014). Furthermore, we aimed at obtaining a sufficient number of trials for detailed EEG analysis. In both sleep groups, exposure to Dutch words occurred during sleep stages 2 and SWS. Sleep was continuously monitored by the experimenter, and the stimulation was interrupted whenever polysomnographic signs of REM sleep, arousal, or awakenings occurred. On average the presentation of Dutch words during sleep was interrupted 5.8 ± 0.4 times.

Recall of vocabulary after the retention interval

During the recall phase, the Dutch words were presented aurally in a randomized order. Subjects had to vocalize the correct German translation. The recall phase was identical to the last learning trial before sleep. As index of memory recall of German translations across the retention interval, we calculated the relative difference between the number of correctly recalled words before and after the retention interval, with the pre-retention memory performance set to 100%.

Sleep EEG

Sleep was recorded by standard polysomnography including electroencephalographic (EEG), electromyographic (EMG) and electrocardiographic (ECG) recordings. EEG was recorded using a high-density 128-channel Geodesic Sensor Net (Electrical Geodesics, Eugene, OR). High-density EEG was used to obtain a reliable estimation of possible topographical distributions to the reactivation-related effects. Impedances were kept below 50 kΩ. Voltage was sampled at 500 Hz and initially referenced to the vertex electrode (Cz). Additionally to the online identification of sleep stages, polysomnographic recordings were scored offline by three independent raters according to standard criteria (Iber et al. 2007). Electrode sites F4, C4 and O2 referenced against average mastoids (electrodes 57 and 100) as well as HEOG (electrode site 1 referenced against electrode site 32) and VEOG (electrode site 25 referenced against electrode site 127) and EMG were used for offline sleep scoring.

Wavelet Analysis

Off-line EEG analysis was realized using Brain Vision Analyzer software (version:2.0; Brain Products, Gilching, Germany). Data were re-referenced to averaged mastoids, low-pass filtered with a cut-off frequency of 30 Hz (roll-off 24 dB per octave) and high-pass filtered with a cut-off frequency of 0.1 Hz (roll-off 12 dB per octave). The EEG data was epoched into 4,000ms segments beginning 1,000ms before stimulus onset. The 1,000ms interval preceding stimulus onset served as baseline and was used for baseline correction. Epochs were categorized based on performance between pre- and post-sleep tests yielding the following categories: We separated later remembered words in "cued gains" and "cued + feedback gains" (i.e., cued words and cued + feedback not remembered before sleep but

correctly recalled after sleep) and "cued hithit", "cued + feedback hithit" words (i.e., cued Dutch words and cued + feedback remembered before and after sleep). Later forgotten words were separated in "cued losses" and "cued + feedback losses" (i.e., cued words and cued + feedback correctly retrieved before sleep but not remembered after sleep) and "cued missmiss", "cued + feedback missmiss" words (i.e., cued Dutch words and cued + feedback not remembered before and after sleep). 4 participants of the correct feedback group and 3 participants of the false feedback group had to be excluded from the EEG analysis due to an insufficient number of trials ($N < 17$). Thus 10 participants of each group entered the EEG analysis.

We analyzed oscillatory activity with regards to the theta (5–7 Hz) and spindle range (12 – 15 Hz). Following Klimesch(Klimesch, 1999) we extracted rather narrow frequency bands for analysis (theta: 5.81-6.03 Hz ; spindle: 12.93-13.25 Hz), thereby reducing the danger that frequency specific effects go undetected. Theta activity was chosen due to a recent finding that successful cueing of vocabulary during sleep was associated with enhanced theta power(Schreiner & Rasch, 2014) while sleep spindles were selected because of their assumed involvement in processes of sleep-dependent memory consolidation. Oscillatory activity frequency bands corresponding to slow wave activity (0.5–4 Hz) were not measured because of the limited number of possible cycles in the short trial length and border effects.

Theta oscillations were analyzed using a Continuous Wavelet Transformation as implemented in Brain Vision Analyzer (complex Morlet waveform, frequency range from 5 to 7 Hz in 10 logarithmic steps, Morlet parameter $c = 7$). For further analysis a frequency range of 5.81-6.03 Hz was used. To avoid edge effects, an interval of 0.4 s at the beginning and the end of the trials was discarded afterwards. In order to obtain the induced power, which is thought to play a role in binding distributed cortical representations(Düzel et al.,

2005), we subtracted the evoked power from each single trial before calculating the time-frequency analysis. After normalization with respect to the prestimulus time window from -300 to -100ms, wavelet analysis was performed on single trials. Subsequently the single trials were averaged. In a first step statistical analysis was performed for a time window of 500 to 800ms after stimulus onset, in order to replicate a recent finding that theta activity in this time frame would vary in relation to cueing success. In a second step we compared activity in 100ms steps, to characterize the timing of potential effects more precisely. The same procedure was performed for sleep spindles (complex Morlet waveform, frequency range from 12 to 15 Hz in 10 logarithmic steps, Morlet parameter c = 7). For further analysis a frequency range of 12.93-13.25 Hz was used. Here too, statistical analysis was initially performed for a time window of 500 to 1000ms after stimulus onset. Afterwards we compared again activity in 100ms steps. To protect against error inflation due to multiple testing of multiple electrodes, we used a false discovery rate (FDR) of $P < 0.05$. Subsequently we averaged the cluster of electrodes surviving the FDR for statistical analysis. For illustration of the results, we present a representative electrode of this cluster. Additionally we were interested in potential effects of the feedback cues during sleep. Therefor we specifically analyzed oscillatory activity with regards to the presentation of the German words in the theta and spindle range. German words entered the category "gains", when the correct pairing was not remembered before sleep but was remembered after sleep. Likewise German words entered the category "losses", when the correct pairing was remembered before sleep but not afterwards. Again potential effects were analyzed in 100ms steps.

Slow oscillations analysis

Artefact-free EEG data, ranging from -1000 to 3000ms with respect to the gain and loss trials, was band-pass filtered between 0.5 – 4.0 Hz (stopband 0.1 and 10 Hz) using a Chebyshev Type II filter (MATLAB, The Math Works Inc, Natick, MA). Slow oscillations were then identified visually at electrode site Fz as well as electrode sites F3 and F4 as waves of a total duration >500ms and a minimal amplitude of 75 μV, starting in a time window between 0 and 800ms post-stimulus.

Statistical Analysis

Data were analyzed using repeated measures analyses of variance (ANOVA). Where appropriate, significant interactions were further evaluated with Fisher's LSD post-hoc tests. The level of significance was set to $P = 0.05$.

Supplementary Information

Supplementary Results:

Behavioral analysis of "Gains" and "Losses"

On the behavioral level, we analyzed "Gains" and "Losses" in both experimental groups (*sleep group I* and *sleep group II*). The results revealed that cueing Dutch words during sleep increased the number of "Gains" and marginally reduced the number of "Losses" in both sleep groups (2-way interaction between the factors "Gains vs. Losses" and "cueing procedure"; $F_{(2,24)} = 20.33$, $P < 0.001$). Additional analysis indicated that in both sleep groups, "Gains" differed significantly between the categories "cued", "cued + feedback" and "uncued" (both $P > 0.02$, see Table 3), with significantly more "Gains" in the cue condition, while "Losses" reached a trend in the reverse direction (*sleep group I*: $P = 0.07$; *sleep group II*: $P = 0.1$).

Supplementary Table 1: Dutch words and German translations used in the learning task (English translations in brackets).

aarde	Erde (ground)	hoek	Ecke (corner)	rubber	Gummi (rubber)
amandel	Mandel (almond)	hout	Holz (wood)	schouder	Schulter (shoulder)
aroma	Geruch (odour)	huis	Haus (house)	schrift	Heft (booklet)
baars	Barsch (harsh)	ijskast	Kuehlschrank (fridge)	schuur	Schuppen (shed)
bagage	Gepaeck (lugagge)	ijzer	Eisen (iron)	servet	Serviette (napkin)
been	Knochen (bone)	inkt	Tinte (ink)	slot	Schloss (lock)
berk	Birke (birch)	kerk	Kirche (church)	sluis	Schleuse (sluice)
beugel	Buegel (bail)	kermis	Jahrmarkt (fair)	snavel	Schnabel (beak)
bezem	Besen (broom)	ketting	Kette (chain)	snuit	Schnauze (snout)
bijl	Axt (axe)	kogel	Kugel (ball)	soep	Suppe (soup)
bloes	Bluse (blouse)	koor	Chor (choir)	speeksel	Speichel (saliva)
boon	Bohne (bean)	kraag	Kragen (collar)	spijs	Brei (puree)
boord	Ufer (border)	kraal	Koralle (coral)	steen	Stein (stone)
borst	Brust (chest)	krijt	Kreide (chalk)	steiger	Steg (gangplank)
boter	Butter (butter)	kuit	Laich (spawn)	stof	Staub (dust)
burcht	Burg (castle)	kus	Kuss (kiss)	stoom	Dampf (stoom)
dak	Dach (roof)	landschap	Landschaft (landscape)	straat	Strasse (street)
dal	Tief (trough)	lat	Latte (lath)	strook	Streifen (stripe)
deur	Tür (door)	lepel	Loeffel (spoon)	tafel	Tisch (table)
dijk	Teich (pond)	lever	Leber (liver)	tegel	Fliese (slab)
doek	Tuch (cloth)	luis	Laus (louse)	trui	Trikot (jersey)
dorp	Dorf (village)	maag	Magen (stomach)	twijg	Zweig (twig)
draad	Faden (twine)	maaltijd	Essen (food)	vaas	Vase (vase)
draak	Drache (dragon)	mees	Meise (chicadee)	vakantie	Ferien (vacation)
droom	Traum (dream)	meeuw	Moewe (gull)	veer	Feder (feather)
duim	Daumen (thumb)	melk	Milch (milk)	vijg	Feige (fig)
eiwit	Eiweiss (protein)	moeras	Sumpf (swamp)	vorst	Frost (cold)
fles	Flasche (bottle)	molen	Muehle (mill)	vuur	Feuer (fire)
folder	Prospekt (brochure)	munitie	Munition (munition)	walm	Qualm (fume)
gat	Loch (hole)	munt	Muenze (coin)	werf	Hof (yard)
gijzelaar	Geisel (hostage)	naam	Name (name)	werktuig	Werkzeug (tool)
gist	Hefe (yeast)	navel	Nabel (navel)	wil	Rad (wheel)
goud	Gold (gold)	olijf	Olive (olive)	wol	Wolle (wool)
graat	Graete (fishbone)	onderdak	Unterkunft (housing)	woordenboek	Wörterbuch (dictionary)
graf	Grab (grave)	paart	Pferd (horse)	worst	Wurst (sausage)
griep	Grippe (flu)	peer	Birne (pear)	zak	Tasche (bag)
grond	Boden (floor)	penseel	Pinsel (brush)	zitkamer	Wohnzimmer (lounge)
hak	Haken (hook)	pols	Puls (pulse)	zeep	Seife (soap)
hei	Heide (heather)	rasp	Reibe (grater)	zoogdier	Saeugetier (mammal)
heuvel	Huegel (hill)	rits	Reihe (row)	zwaan	Schwan (swan)

Supplementary Table 2: Behavioral data of "Gains" and "Losses" for single cues, word-pair cues and uncued words in both experimental groups.

		Cued	Cued + feedback	Uncued	*F*	*P*
Gains						
	Sleep group I	2.35 ± 0.22	1.57 ± 0.34	1.42 ± 0.25	3.83	0.03*
	Sleep group II	2.00 ± 0.11	1.38 ± 0.18	1.46 ± 0.21	4.17	0.02*
Losses						
	Sleep group I	2.50 ± 0.31	3.28 ± 0.47	3.42 ± 0.50	2.83	0.07
	Sleep group II	2.38 ± 0.50	3.38 ± 0.48	3.30 ± 0.44	2.36	0.10
Gains minus Losses						
	Sleep group I	-0.15 ± 0.40	-1.71 ± 0.28	-2.00 ± 0.61	7.91	0.002**
	Sleep group II	-0.38 ± 0.54	-2.00 ± 0.46	-1.84 ± 0.46	7.44	0.003**

Data are means ± s.e.m; "Gains": cued words, which were not remembered in the pre- but remembered in the post-sleep test. "Losses": cued words, which were remembered before sleep, but not after sleep. *: $P < 0.05$; ** $P < 0.01$.

Supplementary Table 3: Significant time-windows comparing "gains" and "losses" for theta and spindle range.

gain / loss Theta	Time window	*t*	*P*-Value
	400-500ms	3.73	0.001*
	500-600ms	4.34	< 0.001*
	600-700ms	4.22	< 0.001*
	700-800ms	3.44	0.003*
	800-900ms	2.77	0.01*
	900-1000ms	2.37	0.028
	1000-1100ms	2.33	0.031
	1100-1200ms	2.66	0.015
	1200-1300ms	2.98	0.023
	1300-1400ms	2.51	0.021
	1400-1500ms	1.99	0.060
	1500-1600ms	1.93	0.68
	1600-1700ms	2.09	0.050
	1700-1800ms	2.35	0.030
	1800-1900ms	2.85	0.01*
	1900-2000ms	3.36	0.003*
	2000-2100ms	3.34	0.003*
	2100-2200ms	3.24	0.004*
	2200-2300ms	3.09	0.006*
	2300-2400ms	2.98	0.008*
	2400-2500ms	2.55	0.01*

gain / loss Spindle	Time window	*t*	*P*-Value
	400-500ms	2.85	0.01*
	500-600ms	3.35	0.003*
	600-700ms	4.04	0.001*
	700-800ms	2.89	0.009*
	800-900ms	2.82	0.01*
	900-1000ms	3.15	0.005*
	1000-1100ms	2.91	0.009*
	1100-1200ms	2.21	0.04
	1200-1300ms	2.91	0.009*
	1300-1400ms	2.76	0.01*
	1400-1500ms	2.46	0.01*
	1500-1600ms	2.50	0.02
	1600-1700ms	2.16	0.04
	1700-1800ms	0.58	0.56
	1800-1900ms	-0.31	0.72
	1900-2000ms	-0.26	0.79
	2000-2100ms	0.91	0.37
	2100-2200ms	0.37	0.57
	2200-2300ms	0.29	0.77
	2300-2400ms	0.60	0.55
	2400-2500ms	-1.61	0.12

* indicate significant P-values surviving FDR correction.

Four

Cueing vocabulary during daytime wake has no effect on memory

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(under review, Somnologie)

Abstract

It is assumed that the beneficial effect of sleep on memory relies on spontaneous reactivation of memories during sleep. We have recently shown that reactivation by re-exposure to previously learned foreign vocabulary cues during sleep benefits vocabulary learning. Cueing foreign vocabulary during active or passive wakefulness at night did not improve memory, suggesting that memory benefits of cueing are sleep-specific. Still, the ineffectiveness of cueing during wakefulness might also be explained by increased tiredness of the participants in this former study. To exclude tiredness as confounding factor, we tested the effect of vocabulary cueing during active and passive daytime wakefulness. We hypothesized that cueing during waking does not improve memory consolidation, even when participants are well rested.

32 subjects learned 120 Dutch-German word pairs. During a 3 hours retention interval, parts of the previously learned Dutch words were replayed again. Subjects of the active waking group (N=16) were distracted from hearing the Dutch words by an n-back task, while subjects of the passive waking group (N=16) were not distracted. After the retention interval memory for word pairs were tested by a cued recall. Replay of Dutch words during daytime wake did not improve later memory for the German translation in both of the waking groups. We observed no difference in recall performance between cued and uncued words, neither in the active waking nor in the passive waking group. Cueing Dutch words during wakefulness does not exert beneficial effects on memory, even when subjects are well rested and under full control of their cognitive capacities. This result gives further evidence that the beneficial effects of cueing are solely sleep specific.

Introduction

Formation of long-term memories requires a phase of consolidation of the memory trace after their encoding (McGaugh, 2000). During consolidation, initially instable memory traces are gradually stabilized involving several cascades of electrochemical and protein-synthesis-dependent processes (Dudai, 2012; Nader & Hardt, 2009; Wixted, 2004). In addition, numerous recent studies now provide evidence that several aspects of memory consolidation are optimally performed during off-line periods like sleep (see (Rasch & Born, 2013), for an overview). While there are different causal mechanisms discussed to underlie the memory function of sleep (Ellenbogen et al., 2006; Tononi & Cirelli, 2014), the active system consolidation hypothesis proposes that during sleep, memory traces are gradually strengthened and integrated into cortical long-term memory based on repeated and spontaneous hippocampal reactivations of newly acquired memories during non-rapid eye movement (NREM) sleep (Diekelmann & Born, 2010; Genzel et al., 2014; Stickgold & Walker, 2013). Importantly, the active system consolidation assumes that these processes are sleep-specific and do not occur during post-learning wakefulness.

Numerous animal studies now provide evidence that memories are indeed spontaneously reactivated during NREM sleep ((O'Neill et al., 2010), for a review): firing patterns and sequential activity of hippocampal place cell activity in rodents observed during wakefulness consistently re-emerge during subsequent Non-REM sleep (O'Neill, Senior, Allen, Huxter, & Csicsvari, 2008; Pavlides & Winson, 1989; Wilson & McNaughton, 1994), frequently associated with characteristic hippocampal sharp-wave ripple (SW-R) events. Such reactivations processes acting during sleep arise also in various other memory related brain regions (i.e., prefrontal cortex, ventral striatum etc. (Ji & Wilson, 2007a; C. M. a Pennartz et al., 2004; Peyrache et al., 2009)), occur in different species from songbirds to humans

(Margoliash, 2010; Peigneux et al., 2004), and appear to be sensitive to relevant and rewarded memories (Lansink et al., 2008; Lansink, Goltstein, Lankelma, McNaughton, & Pennartz, 2009). However in rodents, hippocampal memory reactivations as well as SW-Rs have also been observed during quiet wakefulness after memory encoding (Cheng & Frank, 2008; Kudrimoti et al., 1999). Furthermore, hippocampal replay also occurs during active behavior (e.g., before and after a new run in maze, (Diba & Buzsáki, 2007; Gupta, van der Meer, Touretzky, & Redish, 2010)) and reactivation-association SW-R during post-learning rest are predictive for later memory performance (Dupret et al., 2010). These results suggest that replay activity during wakefulness might be involved in recapitulation and anticipation of behavior and possibly also in subsequent memory consolidation.

In humans the functional significance of memory reactivations during sleep has been repeatedly demonstrated by cueing approaches termed as "targeted memory reactivations" (Oudiette & Paller, 2013). In a first study by Rasch and colleagues (2007) participants learned spatial locations while smelling an odor. During subsequent slow wave sleep (SWS) the odor was given again to trigger the reactivation of the associated memory trace. Re-exposure to the memory-associated odor during post-encoding SWS activated the hippocampus and improved recall performance tested after sleep. The beneficial effect of cueing memories by odors during NREM sleep on consolidation was confirmed in two further studies (Diekelmann et al., 2011; Rihm et al., 2014). In addition, several recent studies extended this finding by successfully using auditory cues like sounds or melodies to reactivate and strengthen individual memories during sleep (Antony et al., 2012; Fuentemilla et al., 2013; Rudoy et al., 2009; Schönauer et al., 2013). For olfactory cues, the beneficial effect of cueing appeared to be specific to sleep, as cueing during post learning wakefulness had either no or even detrimental effects on memory stability (Diekelmann et al., 2011; Rasch et al., 2007).

For auditory cues, results are less clear: While cueing of melodies during post-learning wakefulness did not improve motor memories (Schönauer et al., 2013), re-exposure to sounds during NREM sleep tended to improve memory for sound-place associations (Oudiette et al., 2013; Rudoy et al., 2009). Given further reports of the relevance of spontaneous wake replay for memory processes in humans (Deuker et al., 2013; Fuentemilla, Penny, Cashdollar, Bunzeck, & Düzel, 2010; Peigneux et al., 2006; Staresina, Alink, Kriegeskorte, & Henson, 2013), it is still an open question whether the benefits of auditory cueing on memory consolidation are sleep-specific or similarly occur after cueing during post-learning wakefulness.

In a recent study, we have shown that re-exposure to Dutch words during NREM sleep improved memory for the previously learned German translation (Schreiner & Rasch, 2014). Verbal cues were presented during 3-hours of nighttime sleep. To examine the sleep specificity of the effect, we examined two wake control groups: In the active wake group, verbal cues were presented again during performance of a working memory task, while cueing occurred during quiet resting without any task performance in the passive wake group. The 3-hour wake retention interval occurred at the same time as the sleep interval in the sleep group to exclude circadian influences. In contrast to the sleep group, verbal cueing of Dutch words neither during active nor passive wakefulness after learning improved later memory for the foreign vocabulary. However, as participants stayed awake during nighttime, the ineffectiveness of cueing during wakefulness might alternatively be explained by increased tiredness of the participants in this study. To exclude tiredness as confounding factor, we tested the effect of post-learning cueing of foreign vocabulary during active and

passive daytime wakefulness. We hypothesized that cueing during waking does not improve memory consolidation, even when participants are well rested.

Materials and Methods

Subjects

A total of 32 healthy, right-handed subjects (26 female, mean age = 22.95 ± 0.36) with German mother tongue and without Dutch language skills participated in the study. Thus 16 subjects participated in each of the two experimental groups (i.e., active waking and passive waking group). Age and gender distribution did not differ between the experimental groups (both $P > 0.75$).

None of the participants were taking any medication at the time of the experiment and none had a history of any neurological or psychiatric disorders. All subjects reported a normal sleep-wake cycle and none had been on a night shift for at least 8 weeks before the experiment. On experimental days, subjects were instructed to get up at 7.00h and were not allowed to take in caffeine and alcohol or to nap during daytime. The study was approved by the ethics committee of the Department of Psychology, University of Zurich, and all subjects gave written informed consent prior to participating. After completing the whole experiment participants received 60 Swiss francs (CHF).

Comparison with former study

As results of the present study will be later on compared to results obtained in a recent study (Schreiner & Rasch, 2014), we additionally investigated potential differences between all five experimental groups included in the upcoming analysis concerning age, gender distribution and pre-retention memory performance. There were no significant

differences with regards to age and pre- retention memory performance (age: $F_{(4,76)}$= 1.02, P = 0.41; pre- retention memory performance: $F_{(4,76)}$ = 0.71, P = 0.588). Gender distribution differed significantly between conditions ($F_{(4,76)}$ = 3.52, P = 0.01), indicating that in the present study the gender distribution was less well balanced (26 female, 5 male) than in the experimental groups derived from the former study (24 female, 25 male). Still, since we could not find any effect of gender on memory performance neither in the present study (pre-retention performance: $F_{(1,30)}$ = 0.09, P = 0.75; cueing benefit score: $F_{(1,30)}$ = 0.23, P = 0.62), nor in the former study (pre-retention performance: $F_{(1,47)}$ = 1.53, P = 0.22; cueing benefit score: $F_{(1,47)}$ = 0.31, P = 0.57), this limitation should not severely affect the comparability of results between groups.

With regards to our behavioral findings we directly compared the results of the current studywith the effects of word-re-exposure during the night obtained in our previous study (Schreiner & Rasch, 2014) using planned contrasts. Initially, we compared the cueing benefit score (i.e. correctly recalled and cued words minus correctly recalled and uncued words) of the sleep group against all other wake groups. Subsequently we compared the sleep group's cueing benefit score against both daytime waking groups as well as both nighttime waking groups and finally we directly compared both daytime groups with both nighttime waking groups.

Design and Procedure

In both experimental groups, the beginning of the learning phase was distributed over the entire day (9 a.m. – 3 p.m.). All participants started with the vocabulary-learning task (Dutch-German word-pairs, for a detailed description see section Vocabulary Learning Task). The learning task was followed by a 3-hours retention interval. During the retention

interval, a selection of the previously learned Dutch words was presented again during active or passive waking for a total duration of 90 min (see below for a detailed description of the reactivation phase). After the retention interval, recall of the vocabulary was tested in both experimental groups.

Vocabulary Learning Task

The vocabulary-learning task consisted of 120 Dutch words and their German translation, randomly presented in three learning rounds (please refer to the supplementary information of (Schreiner & Rasch, 2014) for a list of all vocabulary pairs). Dutch words were presented aurally (duration range 400-650ms) via loudspeakers (70 dB sound pressure level). In the first learning round, each Dutch word was followed by a fixation cross (500ms) and subsequently by a visual presentation of its German translation (2,000ms). The intertrial-interval between consecutive word-pairs was 2,000-2,200ms. The subjects were instructed to memorize as many word-pairs as possible. In a second round the Dutch words were presented again followed by a question mark (ranging up to 7 seconds in duration). The participants were instructed to vocalize the correct German word or to say, "next" (German translation: "weiter"). Afterwards, the correct German translation was shown again for 2,000ms, irrespective of the correctness of the given answer. In the third learning round, the cued recall procedure was repeated without any feedback of the correct German translation. Recall performance of the third round (without feedback) was taken as pre-retention learning performance. In the third round, participants recalled on average 62.41 $\pm$ 1.71 words (range 44 – 84 words) of the 120 words correctly, indicating an ideal medium task difficulty (recall performance 52%) without any danger of ceiling or floor effects. We observed no difference in pre-retention memory performance between the two experimental

groups (main effect of "group": $F_{(1,30)}$ = 0.57; P = 0.45), no difference in pre-retention memory performance between later cued and uncued words (main effect "cueing": $F_{(1,30)}$ = 0.77; P = 0.38) and no interaction between "group" and "cueing" ($F_{(1.30)}$ = 0.47; P = 0.49; see Table 1 for descriptive statistics).

Reactivation of vocabulary

During the 3-h retention interval, Dutch words were presented aurally without the German translation. The presentation occurred via loudspeakers (50 dB sound pressure level). Out of the 120 words learned before the retention interval, 60 words were cued and 60 were not cued during the subsequent retention interval. The 60 cued words consisted of 30 words that participants remembered during the pre-retention learning phase (cued hits) and 30 words that participants did not remember before the retention interval (cued misses). The words were individually and randomly chosen for each participant using an automatic MATLAB algorithm. In addition, 30 new words were presented during the retention interval that had not been included in the pre-retention learning list, serving as control stimuli. Thus, in total 90 Dutch words were presented during the retention interval. Presentation occurred every 2.800-3.200ms in a randomized order for a total of 90 minutes, resulting in 10-11 exposures to each word. In the active waking group, cueing of Dutch words occurred during performance on a computerized n-back task. The 3-hour wake retention interval was divided into 30 min periods. In the first, third and fifth 30 min period, participants performed on the n-back task (including a total of 27 67-s blocks of 0-back, 1-back and 2-back blocks, in a randomized order, for more details see task description). Subjects were instructed to focus on the task and were given feedback on accuracy after each 30 min period. While subjects accomplished the n-back task, Dutch words were replayed. Between the three blocks of

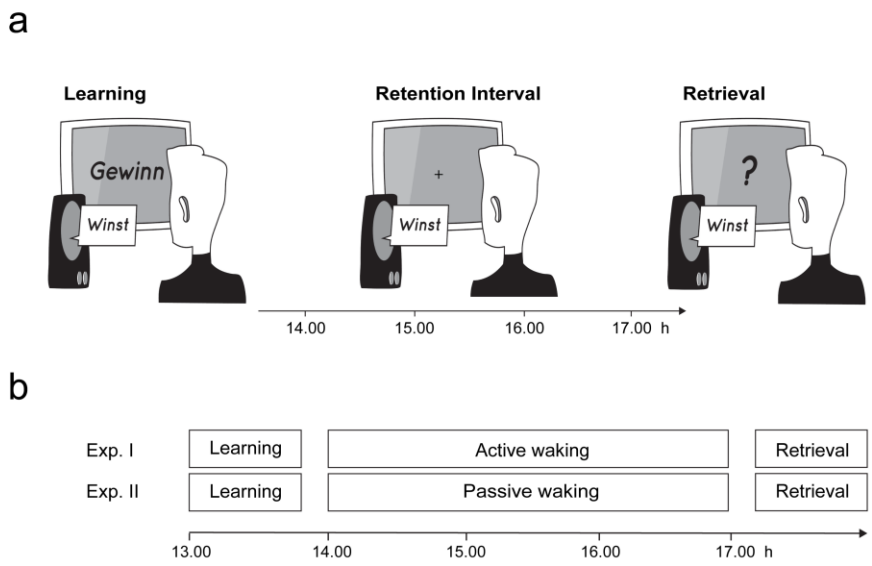
word reactivation, subjects completed questionnaires and played an online computer game (Bubble-shooter). In the passive waking group, Dutch words were replayed during passive waking of the participants, allowing full attention on the replayed Dutch words. Participants were re-exposed to the Dutch words in the first, third and fifth 30 min period of the 3-h retention interval. They were instructed that they would hear some of the Dutch words again and should attentively listen to the words. In the remaining 30 min-periods, the participants performed on the n-back task and filled out questionnaires, without any auditory stimulation.

Recall of vocabulary after the retention interval

During retrieval, the Dutch words were presented via loudspeaker in a randomized order. Additionally to the 120 words which were learned before the retention learning, the 30 control words from the reactivation phase and 30 entirely new words were tested. Initially participants had to indicate whether the word was old (part of the learning material) or new. If the current word was recognized as old, subjects were asked to vocalize the German translation. As index of memory recall of German translations across the retention interval, we calculated the percentage of correctly recalled words at retrieval relative to correctly recalled words at learning, with the level of learning set to 100%.

For recognition memory of Dutch words, we calculated the sensitivity index d' (i.e., Hits/True Positives – False Positives/True Negatives) according to signal detection theory. Proportions of 0 and 1 were replaced by 1/2N and 1-1/2N, respectively, with N representing the number of trials in each proportion (i.e., N = 60, see (Macmillan & Creelman, 2005)). The memory indices for cued recall and recognition were calculated separately for cued and uncued words.

Figure1. Experimental procedure



Experimental procedure. (a and b) Participants studied 120 Dutch–German word pairs during daytime. During the retention interval, 90 Dutch words (30 prior remembered, 30 prior not remembered and 30 new words) were repeatedly presented again. Cueing of vocabulary occurred during performance of a working memory task (active waking), or during rest (passive waking). After the retention interval, participants were tested on the German translation of the Dutch words using a cued recall procedure.

n-Back Test

Subjects of both waking groups accomplished intermixed 0-, 1- and 2- back versions of the n-back working memory task (Gevins & Smith, 2000). In this task different letters appear successively in the center of the screen. In the 0-back version subjects had to press a key whenever the letter "x" appeared on the screen. In the 1-back version subjects had to respond to a letter repetition (h-f-f-k), while the 2-back version requires subjects to respond to a letter repetition with one intervening letter (h-f-s-f).

Results

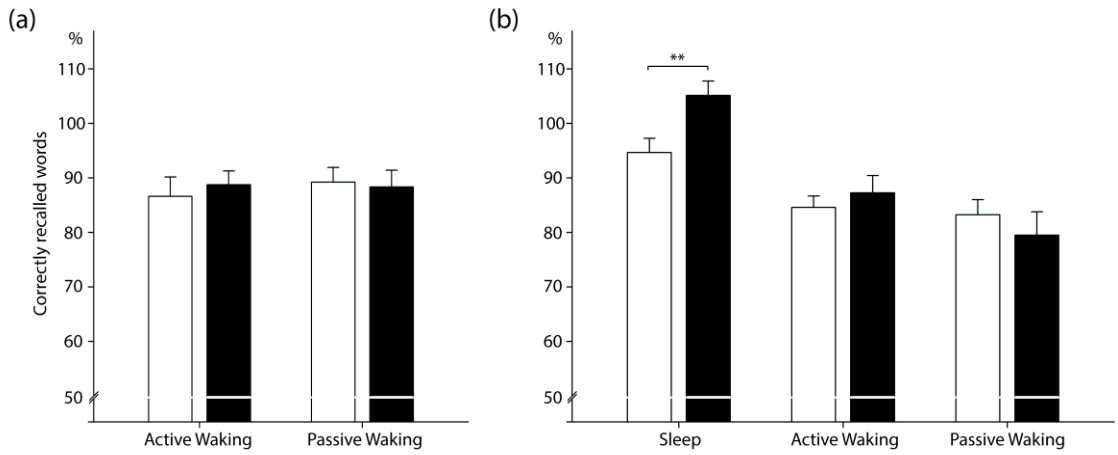
Effects of verbal cueing during wake on cued recall performance

As expected, re-exposure to Dutch words after learning during daytime wakefulness did not improve later memory for the German translation in both of the waking groups. Overall after cueing during daytime wakefulness, participants recalled 88.54 ± 1.96 % of the cued words and 87.91 ± 2.21 % of the uncued words (main effect of "cueing": $F_{(1,30)}$ = 0.041; P = 0.84; please note that the retrieval performance is indicated as percentage of recalled German translations with performance before the retention interval set to 100%.). The effect was similarly lacking after re-exposure to Dutch words during active as well as passive waking (no interaction between "cueing" and "group"; $F_{(1,30)}$ = 0.23; P = 0.62, see Table 1 for descriptive values). In order to elucidate potential influences of the time of day on the behavioral results, we correlated the onset time of the experiment with the pre-retention performance and the cueing benefit score. Both correlations remained non-significant (pre-retention performance: r = -0.05, P=0.77; cueing benefit score: r= -0.26, P=0.14), safely excluding potential timing influences.

In addition, we directly compared the results of the current study with the effects of word-re-exposure during the night obtained in our previous study (Schreiner & Rasch, 2014). In order to assess the influence of different brain states (i.e. wake/sleep), we conducted planned contrast on a cueing benefit score (i.e. correctly recalled and cued words minus correctly recalled and uncued words). In a first step we compared the sleep group against all other wake groups. The benefit score of the sleep group differed significantly from the overall wake group score (t_{76} = 2.75; P = 0.01), indicating a general sleep specificity concerning the beneficial effects of cueing. Furthermore we compared the sleep group's cueing benefit score against both daytime waking groups as well as both nighttime waking groups, to prove that

the beneficial effects of cueing during sleep differ from waking effects irrespective of day- and nighttime, respectively. Again the benefit score of the sleep group differed significantly from the waking groups score, both for the daytime waking groups (t_{76} = 2.17; P = 0.03) and for the nighttime waking groups (t_{76} = 2.68; P = 0.01). Thus irrespective of whether cueing happened during daytime or nighttime waking, the beneficial effects were dependent on sleep. To further demonstrate that cueing during wakefulness has no beneficial effect on memory irrespective of day/nighttime we directly compared both daytime groups with both nighttime waking groups. As expected the benefit score did not differ (t_{76} = 0.17; P = 0.89), further demonstrating that the lacking effect of cueing during wakefulness is not dependent on the time of day.

Figure 2. Behavioral results



Behavioral results. **(a)** No enhancing effects of cueing during daytime on later memory retrieval occurred in both waking groups. Memory for cued word pairs (black bar) did not differ from uncued pairs (white bar). **(b)** This figure was adopted from (Schreiner & Rasch, 2014) illustrating the behavioral results of cueing during sleep and nighttime waking. In the sleep group, memory for cued word pairs (black bar) was significantly improved when compared with uncued pairs (white bar). No enhancing effects of cueing on later memory retrieval occurred in both waking control groups. Additionally planned contrasts revealed that the beneficial effects of cueing on memory during sleep differed significantly from both daytime waking groups as well as both nighttime waking groups. Retrieval performance is indicated as percentage of recalled German translations with performance before sleep set to 100%. Values are mean ± SEM. **P ≤ 0.01

Effects of verbal cueing during wake on recognition memory

As with the results concerning the cued recall, re-exposure to Dutch words did not have any effect on recognition memory in either waking group. There was no difference in recognition performance between cued and uncued words (d' = 1.70 ± 0.15 vs. d' = 1.68 ± 0.14; main effect of "cueing": $F_{(1,30)} = 0.21$; $P = 0.64$). In addition, we did not observe any interaction between "cueing" and "condition" ($F_{(1,30)} = 0.37$; $P = 0.54$; see Table 1 for descriptive statistics and absolute values). Thus recognition memory performance was as well unaffected by the cueing procedure. In addition, we explored whether the presentation of new words during the retention interval resulted in deteriorated recognition performance as compared to memory performance for the entirely new words, which were presented during recognition testing for the first time. As one might expect presentation of new words during the retention interval worsened recognition memory (cued new words % Hits: 73.02 ± 2.62; entirely new words % Hits: 83.64 ± 2.81; $t = -5.21$, $P < 0.001$).

In our previous study (Schreiner & Rasch, 2014), cueing did also not affect recognition performance neither for the sleep group nor the two nighttime wake groups. Also when including the current two daytime wake groups, none of the calculated planned contrasts reached significance (all $p > 0.28$), indicating that effects of cueing on recognition performance were not altered by the circadian time of the retention interval.

Table 1. Overview of memory performance

		Cued	Uncued	*t*	*P*
Active waking group					
Cued recall	*Learning*	30	32.62 ± 2.67	-0.98	0.34
	Retrieval	26.62 ± 0.76	27.56 ± 2.02	-0.54	0.59
	Change	-3.37 ± 0.71	-4.43 ± 1.06	0.85	0.41
	% Change	88.75 ± 2.54	86.61 ± 3.55	0.51	0.61
Recognition	*Hits*	49.50 ± 0.88	48.81 ± 1.58	0.64	0.53
	% Hits	82.50 ± 1.48	80.35 ± 2.64		
	d'	2.01 ± 0.15	2.02 ± 0.16	-0.12	0.89
Passive waking group					
Cued recall	*Learning*	30	30.31 ± 2.01	-0.15	0.86
	Retrieval	26.50 ± 0.92	26.68 ± 1.65	-0.12	0.90
	Change	-3.50 ± 0.92	-3.43 ± 1.00	-0.04	0.96
	% Change	88.33 ± 3.08	89.21 ± 2.73	-0.19	0.84
Recognition	*Hits*	48.31 ± 1.07	46.75 ± 1.49	1.02	0.32
	% Hits	80.52 ± 1.79	77.91 ± 2.49		
	d'	1.61 ± 0.11	1.53 ± 0.11	0.66	0.51

Data are means ± s.e.m; Numbers indicate absolute or relative values of correctly recalled or recognized words that where presented during the retention interval (cued words, 60 in total) or not (uncued words, 60 in total). For cued recall testing, number of correctly recalled words during the learning phase before and the retrieval phase after the retention interval are indicated. Change (% Change) refers to the absolute (relative) difference in performance between learning and retrieval phases. Hits (%Hits) refers to the absolute (relative) number of correctly recognized words as "old" (since %Hits = Hits*100/60, statistics are redundant). The sensitivity measure d' reflects recognition performance according to signal detection theory based on the proportion of Hits and False Alarms (Macmillan & Creelman, 2005) .

Table 2. Baseline performance

	Active waking	Passive waking	Sleep*	Active waking*	Passive waking*
pre-retention performance	62.21 ± 2.67	60.31 ± 1.93	63.06 ± 2.50	60.70 ± 2.71	58.17 ± 1.79

Data are means ± s.e.m; Numbers indicate absolute values of correctly recalled words before the retention interval. * indicates experimental groups derived from a previous study (Schreiner & Rasch, 2014).

Discussion

Here we show that cueing foreign vocabulary during a wake retention interval after learning has no beneficial effect on recall performance, when cueing takes place during rested wakefulness. Our current findings are in line with results of our recent study (Schreiner & Rasch, 2014), indicating that cueing Dutch vocabulary during the retention interval improves recall of the newly-learned German translations only when cueing occurres during post-learning sleep, but not during post-learning wakefulness. Importantly, in our former study participants had to stay awake during the night, which might have prevented positive effects of cuing due to the potential participants sleepiness. Here we demonstrate that cueing memories during wakefulness during the day is still ineffective, even when participants are well rested and at the height of their cognitive capability. Furthermore the availability of attentional resources had no bearing on the obtained results since unattended (active wake group) as well as attended cues (passive wake group) failed to improve later recall performance.

The missing effect of cueing during wakefulness is well in line with the active system consolidation hypothesis (Rasch & Born, 2013), which assumes that the enhancing effect of sleep on memory consolidation critically relies on spontaneous memory reactivations during sleep. The model posits that the state of NREM sleep offers ideal conditions for a strengthening of memories after their reactivation, due to the presence of slow oscillations, sleep spindles, a minimal cholinergic tone and the exclusion of external interference (see also (Cordi, Diekelmann, Born, & Rasch, 2014; Diekelmann et al., 2011)). In contrast, memory reactivations during wakefulness are ineffective in strengthening memories due to the absence of the above mentioned factors, or possibly even render already consolidated memories again in a labile state as proposed by the reconsolidation theory. (Rasch & Born,

2013).

Evidence for the assumption that reactivations during sleep play a functional role for strengthening memories during sleep is provided by a constantly growing number of experiments demonstrating that increasing reactivation during sleep by cueing improves later memory recall after sleep (Oudiette & Paller, 2013). However, the assumption of a sleep-specific role of reactivations for memory consolidation is less clear. Several studies in rodents have shown that replay of hippocampal place cells occurs similarly during quiet waking of the animal as well as before and after task performance during wakefulness (O'Neill et al., 2010). In addition, a growing number of recent studies using functional magnetic resonance imaging (fMRI) (Deuker et al., 2013; Peigneux et al., 2006; Tambini & Davachi, 2013) reported spontaneous reactivations of learning related activity during waking rest, which are predictive for later memory performance . Thus, spontaneous memory reactivations exist during wakefulness, and they might even be involved in the process of memory consolidation during wakefulness.

In support for a functional role of reactivations during post-learning wakefulness, one afternoon nap study by Oudiette and colleagues [26] reported beneficial cueing effects during waking. In this experiment participants had to learn an object location task, where each object was presented in parallel with a characteristic sound. Furthermore each object belonged to a high value or a low value category, indicating subsequent payoff. During a subsequent retention interval either of sleep or wakefulness, parts of the low value sounds were replayed again. Here cueing memories exerted a beneficial effect on subsequent memory performance, when cueing took place during wakefulness, while participants performed a working memory task. In contrast to this finding, most other studies (including our own) using targeted memory reactivation did not observe memory benefits after cueing

during post-learning wakefulness. Thus, it might be possible that the reported cueing benefit during wakefulness reported by Oudiette et al. is linked to specific features of the task used in this study. For example the expectancy of reward might have increased the beneficial effects of targeted memory reactivation, as reward-associated memories are preferentially reactivated (Lansink et al., 2008, 2009; Peyrache et al., 2009). Future studies will be needed to determine whether reward expectancy is critical for cueing benefits on memory consolidation during post-learning wakefulness.

In sum, our results add evidence to the notion that the benefits of memory reactivations for memory strength and later recall are sleep specific and do not occur during nighttime or daytime wakefulness. Furthermore, recent studies also indicate that targeted reactivations during REM sleep are similarly ineffective (Cordi et al., 2014; Sterpenich et al., 2014), suggesting that reactivations strengthen memories exclusively during NREM sleep. Thus, even though memory reactivations might occur during passive and active wakefulness as well as NREM and REM sleep, the consequence for memory strength seems to differ between these brain states. The reasons and underlying mechanisms why only reactivations during NREM sleep strengthen memories are still not completely clear. The absence of external interfering input as well as the occurrence of slow waves and sleep spindles are probably necessary conditions for a strengthening effect of reactivations on memories. In addition, the low cholinergic tone characteristic for NREM slight might be a prerequisite for disinhibiting hippocampal-neocortical feedback loops (Hasselmo, 2006), allowing a successful integration and strengthening of reactivated memories into cortical knowledge networks. Still, further studies will have to identify the underlying neural mechanisms and critical factors of the beneficial role of reactivation during NREM sleep on long-term memory formation.

Five

Cueing vocabulary during sleep increases theta activity during later recognition testing

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(under review, International Journal of Psychophysiology)

Abstract

Neural oscillations in the theta band have repeatedly been implicated in successful memory encoding and retrieval. Several recent studies have shown that memory retrieval can be facilitated by reactivating memories during their consolidation during sleep. However, it is still unknown whether reactivation during sleep also enhances subsequent retrieval-related theta oscillations. We have recently demonstrated that foreign vocabulary cues presented during sleep improve later recall of the associated translations. Here we examined the effect of cueing foreign vocabulary during sleep on theta-activity (5 – 7 Hz) during subsequent recognition testing after sleep. We show that those words, which were replayed during sleep after learning (cued words), elicited stronger parietal theta activity during recognition as compared to non-cued words. The reactivation-induced increase in theta oscillations during later recognition testing might reflect a strengthening of individual memory traces by cueing during sleep.

Introduction

Extensive evidence has accumulated that memory formation and consolidation during wakefulness and sleep are heavily based on neural oscillatory synchronisation (Diekelmann & Born, 2010; Fell & Axmacher, 2011a). During wakefulness, oscillatory theta activity has been consistently linked to the encoding and retrieval of new declarative information (Nyhus & Curran, 2010a). Theta activity seems to be related to long-term potentation and synaptic plasticity, thereby facilitating the encoding of new memories (Huerta & Lisman, 1995; Hyman, Wyble, Goyal, Rossi, & Hasselmo, 2003). Concerning the retrieval of declarative memories oscillatory theta is assumed to drive the hippocampus-dependent reinstatement of individual memories in parietal cortex (Nyhus & Curran, 2010a).

In line with this assumption several studies focusing on recognition memory, reported enhanced parietal theta power in association with correctly identified studied (old) words when compared with correctly rejected non-studied (new) words (Jacobs, Hwang, Curran, & Kahana, 2006; Kim et al., 2012). Based on these results parietal theta activity is assumed to reflect the strength of episodic memory traces (Klimesch et al., 2006).

After their encoding, memories are consolidated during subsequent sleep, and it is assumed that the beneficial effect of sleep on memory is due to spontaneously occurring hippocampal memory reactivations during Non rapid-eye movement (Non-REM) sleep (Rasch & Born, 2013). A causal role of those memory reactivations is supported by studies showing that experimentally inducing reactivations during Non-REM sleep by using associated memory cues benefits memory consolidation and activates hippocampal brain regions during sleep (Dongen, Takashima, et al., 2012; Oudiette & Paller, 2013; Rasch et al., 2007; Rudoy et al., 2009; Schönauer et al., 2013). In spite of the strong evidence for a behavioral effect of cueing during sleep on later memory retrieval, it is largely unknown whether cueing also affects retrieval-related brain responses after sleep.

In a very recent study, we have shown that a replay of prior learned Dutch words during sleep enhances the recall of the German translations of those words (Schreiner & Rasch, 2014). EEG was further recorded during recognition testing after sleep. We hypothesize that replaying Dutch words during sleep also increases theta oscillations during recognition, indicating a strengthening of memory traces by cueing during sleep on a neural level.

Materials and Methods

The data were taken from Schreiner & Rasch (2014). Detailed information about participants, stimuli, task, data acquisition sleep data and behavioral results can be found in Schreiner & Rasch (2014). 15 healthy, right-handed subjects (8 female) with German mother tongue and without Dutch language skills participated in the study. Data of one subject had to be discarded due to technical problems. The study was approved by the ethics committee of the Department of Psychology, University of Zurich, and all subjects gave written informed consent prior to participating.

The learning phase started at 22.00h. Participants were confronted with 120 Dutch German word pairs. After completing the learning task, participants slept for 3 hours. During Non-REM sleep 60 out of the 120 Dutch words learned before were replayed again via loudspeaker (50 dB). Participants were awakened at ~ 2.15h and recall of the vocabulary was tested afterwards. In the recognition phase the 120 Dutch words included in the pre-retention learning list were presented again aurally intermixed with 60 entirely new Dutch words. After listening to each word participants had to indicate whether the word was old (part of the learning material) or new. If the current word was recognized as old, they were asked to give the German translation (for an overview concerning the behavioral results see supplementary Table 1).

EEG recording and analysis

EEG was recorded using a high-density 128-channel Geodesic Sensor Net (Electrical Geodesics, Eugene, OR, USA). Impedances were kept below 50 kΩ. Voltage was sampled at 500 Hz and initially referenced to Cz. Off-line EEG analysis was realized using Brain Vision Analyzer software (version:2.0; Brain Products, Gilching, Germany). Data were re-referenced to averaged mastoids, low-pass filtered at 30 Hz and high-pass filtered at 0.1 Hz.

Artifact-affected trials meeting the following criteria were labeled and finally rejected: voltage values exceeding +/-75μV or a voltage drift of more than 75 μV. Eye blinks and movements were corrected using independent component analysis (Jung et al., 1998). For artifact free trials theta oscillations (5–7Hz) were analyzed using a Continuous Wavelet Transformation (complex Morlet waveform, frequency range from 5 to 7 Hz in 10 logarithmic steps, Morlet parameter c = 7). In order to avoid edge effects, trials entering the wavelet transform were segmented from −0.7 to 1.9 s with respect to stimulus presentation. An interval of 0.4 s at the beginning and the end of the trials was discarded afterward. A total of both induced and evoked activity was calculated by performing the wavelet analysis on single trials, after normalization with respect to the prestimulus time window from −300 to −100 ms. Subsequently, the resulting single-trial frequency spectra were averaged. This procedure provides the overall power of a given frequency range. To obtain the induced power, which is thought to play a role in binding distributed cortical representations (Düzel et al., 2005), we subtracted the theta effects of the average ERP (evoked power) from each single trial before calculating the time–frequency analysis and averaging the single trials individually and per group for correct rejections (correctly identified new words) and hits (correctly identified old words) and subsequently for cued and uncued hits (correctly identified old words replayed respectively not replayed during sleep) (Klimesch et al., 1998).

To protect against error inflation due to multiple testing of multiple electrodes, we used a false discovery rate (FDR) of $P < 0.05$. For illustration, we present the oscillatory results of the electrode with the highest significance.

Statistical Analysis

Data were analyzed using repeated-measures analyses of variance (ANOVA). Where appropriate, significant interactions were further evaluated using t-tests.

Results

Behavioural results

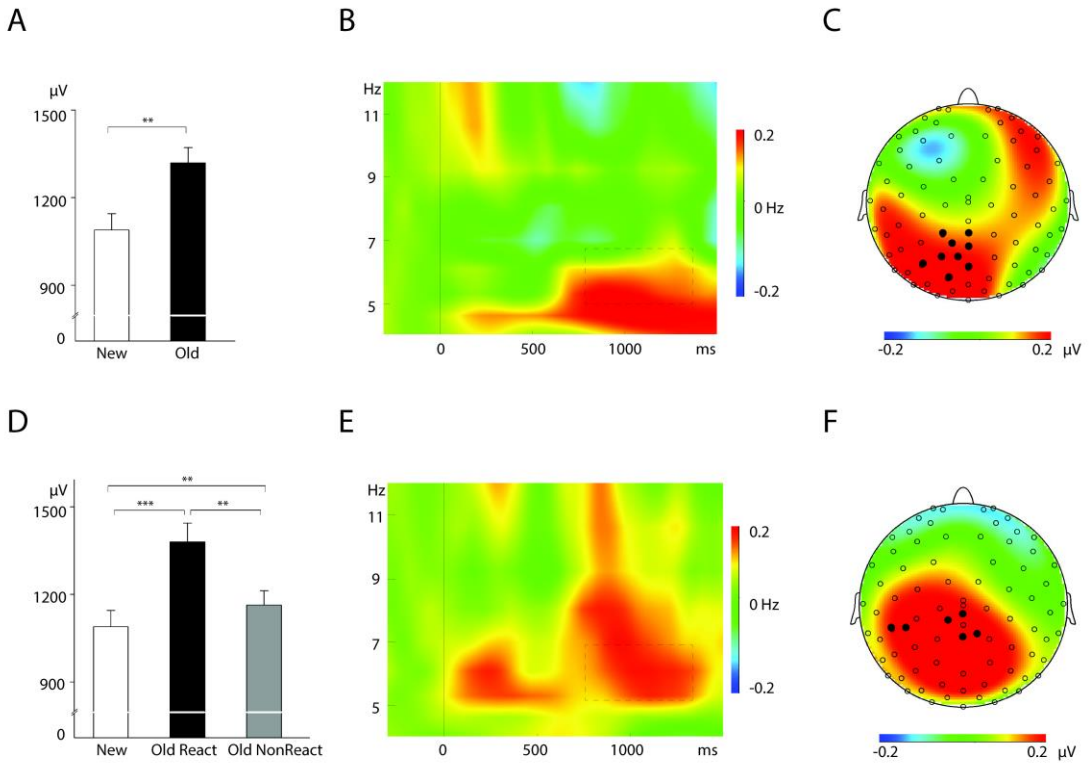
As reported previously, cueing during sleep increased cued recall of the associated German translation of the Dutch words after sleep (see Schreiner & Rasch, 2014). In contrast, recognition performance of the Dutch words did not differ between cued and uncued words ($p > 0.8$).

Oscillatory results

Initially we examined whether successful recognition in general is accompanied by enhanced theta power independent of cueing during sleep. We compared induced theta power associated with correctly identified old and new words, irrespective of previous replay. As expected, induced theta power differed significantly between conditions, with an increased theta power for old words at left parieto – occipital electrode sites in a time window of 800 – 1400ms after stimulus onset (electrode Pz: $t_{14} = 4.46$, $P = 0.001$; further electrodes reaching significance: E60, E65, E66, E67, E70, E71, E72, E75). To specifically determine potential effects of cueing on theta power, we further divided the total number of recognized old words into old words, which were replayed during sleep (cued) and old words not replayed during sleep (uncued). The three categories (cued old words, uncued old words and new words) differed significantly with regards to theta power (electrode Pz: $F_{2,26} = 10.02$; $P = 0.001$), with the cued old words depicting the strongest theta power in a time window of

800 – 1400 ms (cued old vs. uncued old: $t_{14} = 4.69$, $P = 0.001$; cued old vs. new: $t_{14} = 3.05$, $P = 0.009$). This effect had a stable parieto – occipital topography.

Figure 1. Oscillatory results



Oscillatory theta power (5 – 7 Hz) recorded during recognition was computed for hits (words correctly identified as old) and correct rejections (words correctly identified as new). To determine potential effects of cueing on theta power, the total number of recognized old words was divided into old words, which were replayed during sleep (cued) and old words not replayed during sleep (uncued).(**a + b**) When comparing theta power associated with the presentation of old and new words, increased theta power emerged for old words particularly at left parieto – occipital electrode sites in a time window of 800 – 1400 ms after stimulus onset (representative electrode Pz). (**c**) Scalp map representing the topographical distribution for the difference between "Old" and "New" in the time window between 800 and 1400 ms. (**d)** Induced theta power for "OldCued" ,"OldUnced" and "New" **. (e**) Induced theta power for the difference between "OldCued" and "OldUnced" (electrode Pz), indicating a distinct increase in induced theta power for words which were replayed during sleep. (**f**) Scalp map depicting the parietal distribution of theta power increase for "OldCued" relative to "OldUncued" in the time window between 800 and 1400 ms. Data are presented as mean ± s.e.m. *** $P \leq 0.001$, ** $P \leq 0.01$.

Discussion

Our results demonstrate that cueing prior learned foreign vocabulary during sleep leads to enhanced theta activity in a subsequent recognition memory task. Theta activity as been repeatedly shown to be enhanced during successful memory retrieval (Nyhus & Curran, 2010a), particularly in parietal regions (Jacobs et al., 2006) and might reflect or associated with the strength of a memory trace (Klimesch et al., 2006). According to the activity system consolidation theory, spontaneous hippocampal memory reactivations during SWS critically contribute to the memory-strengthening effect of sleep after learning. Several recent studies have successfully used memory-associated odors, sounds, or vocabulary (Rasch et al., 2007; Rudoy et al., 2009; Schreiner & Rasch, 2014) to target reactivation during sleep and thereby improve retrieval performance on the behavioral level. Our results add to this support by showing that targeted memory reactivation during sleep also increases oscillatory neural markers in the theta-band in parietal brain areas, possibly indicating an increase in memory strength by cue during sleep. Interestingly, given that cueing during sleep behaviorally only exerted beneficial effects on memory performance acquired by cued recall but not on recognition memory, theta activity associated with recognition memory processes seems to represent a more fine-grained measure to index memory strength. To determine the exact source of the obtained activity pattern, future studies should employ other brain imaging techniques (e.g. combined EEG/fMRI recordings), given the potential role of the hippocampus concerning memory consolidation during sleep, as well as its relationship to theta activity and memory retrieval.

Supplementary Information

Supplementary Table 1. Overview of recognition memory performance

		Cued	Uncued	*t*	*P*
Recognition	*Hits*	52.40 ± 0.98	51.20 ± 1.57	1.33	0.80
	% Hits	87.33 ± 1.62	85.33 ± 2.62		
	d'	2.32 ± 0.15	2.32 ± 0.17	0.00	0.99

Data are means ± s.e.m; Numbers indicate absolute or relative values of correctly recognized words that where presented during the retention interval (cued words, 60 in total) or not (uncued words, 60 in total). Change (% Change) refers to the absolute (relative) difference in performance between learning and retrieval phases. Hits (%Hits) refers to the absolute (relative) number of correctly recognized words as "old" (since %Hits = Hits*100/60, statistics are redundant). The sensitivity measure d' reflects recognition performance according to signal detection theory based on the proportion of Hits and False Alarms (Macmillan & Creelman, 2005) . *$P < 0.05$; ** $P < 0.01$.

SIX

Summary

Discussion

Outlook

In the present thesis a number of studies have been presented closely investigating the effects of reactivating memories using vocabulary cues. One main focus of this work was to examine whether such highly complex cues as foreign vocabulary are generally capable of inducing memory reactivations during sleep, thereby enhancing memory performance. Additionaly we investigated the neural correlates of successful reactivation processes. Furthermore, we described the effects of replacing single cues by congruent and incongruent vocabulary pairs on oscillatory activity during sleep and later memory performance. We determined in depth the sleep specificity of auditory cueing procedures and examined whether cueing during sleep would leave a trace in subsequent oscillatory activity during recognition testing. In what follows, a brief summary of the main findings of the reported studies will be given, before discussing them in relation to the current literature. Finally future directions will be envisaged.

6.1 Summary

In Chapter 2 we explored whether verbal cueing during Non-REM sleep would improve vocabulary learning. Indeed, replay of Dutch words during sleep improved later memory for the German translation of the cued words when compared with uncued words. Interestingly recall of uncued words was similar to an additional group receiving no verbal cues during sleep, indicating that the beneficial effects of cueing during sleep might exceed the normal consolidation effects of sleep on memory. Additionally, verbal cueing did not benefit memory during active and passive waking. On a neural basis, we found that successful verbal cueing during NonREM sleep was associated with a pronounced frontal negativity in ERP's, a higher frequency of frontal slow waves as well as a cueing-related increase in oscillatory theta power. Thus, here a first step was made by demonstrating that

verbal cues are capable of inducing memory reactivations during sleep and by obtaining first results concerning neural patterns associated with successful cueings during sleep.

Based on the results of Chapter 2 that replay of single vocabulary cues during sleep improves later memory performance, we investigated in Chapter 3 whether replay of congruent and incongruent Dutch German word pairs during sleep would lead to enhanced or deteriorated recall performance, respectively. We replayed prior learned Dutch words either as single word cues or including congruent and incongruent feedback in two sleep groups. While cueing of single words enhanced memory performance, thereby replicating our earlier findings, cue + feedback replay did not exert any behavioural effect, independent of content. On a neural basis, successful single word cueing during NonREM sleep was associated with enhanced oscillatory theta and spindle activity. Interestingly, these neural patterns vanished when feedback cues were presented, indicating that the presentation of a second stimulus blocked reactivation-associated processes, thereby possibly leading to the lack of behavioral effects.

While cueing during active or passive wakefulness at night did not improve memory, the ineffectiveness of cueing during wakefulness might as well be explained by increased tiredness of the participants of the study described in Chapter 2. Thus, in order to drill down on the sleep specificity of our results we investigated in Chapter 4 the effect of vocabulary cueing during active and passive daytime wakefulness. Again replay of Dutch words did not improve later memory for the German translation in both of the waking groups. Hence, there was no difference during daytime in recall performance between cued and uncued words, neither in the active nor in the passive waking group. Accordingly, this result supplies further evidence that the beneficial effects of cueing seem to be solely sleep specific.

In Chapter 5 we investigated whether cueing during Non-REM sleep would lead to enduring changes in oscillatory theta power during subsequent recognition testing. We focused on theta activity since neural oscillations in the theta band have been repeatedly implicated in successful memory encoding and retrieval. We show that those words, which were replayed during sleep after learning, elicited stronger parietal theta power during recognition as compared to non-cued words. This result suggests that the reactivation-induced increase in theta activity might reflect a strengthening of individual memory traces by cueing during sleep.

6.2 Discussion

As outlined in Chapter 1 the active system consolidation theory proposes that the memory function of sleep relies on spontaneous hippocampal memory reactivations during SWS, leading to memory reactivations in the neocortex and thereby promoting the stabilization and integration of those memories (Diekelmann & Born, 2010). Accordingly, the functional significance of those memory reactivations has been repeatedly demonstrated by cueing studies. Specifically, several studies have successfully used memory-associated odors, sounds, or even melodies (Antony et al., 2012; Rasch et al., 2007; Rudoy et al., 2009) to cue and strengthen memories during sleep. The results described in Chapter 2 and 3, namely that cueing of single Dutch words during sleep improved later memory for the German translations are consistent with the active system consolidation hypothesis and fit well to the existing literature. Furthermore, they match to the predictions of the Distributed Cohort Model (Davis & Gaskell, 2009a), which assumes as critical role of memory reactivations for word learning.

Importantly, our results go an important step beyond these previous results by

demonstrating that also highly complex cues such as foreign vocabulary can be successfully used to reactivate memories during sleep, leading to an enhanced memory for vocabulary the next day. The fact that we found corresponding effects concerning single word replay in two independent studies, including three experimental groups, indicates the stability of our cueing procedure. Even the amount of performance improvement, namely 10%, was stable between all experimental groups and goes well with the usually reported margins of 5-15% (Diekelmann, 2014).

Interestingly results in Chapter 2 suggest that the enhancing effect of vocabulary cueing during sleep exceeds the natural consolidation effects of sleep on memory, since recall of uncued words in the cueing sleep group was equal to memory performance of sleeping control participants who did not receive any cues during sleep. Hence, verbal cueing during sleep appears to benefit later recall of cued memory associations without disturbing ongoing consolidation processes during sleep. Since other studies, dealing with the cueing of procedural memories, reported that cueing resulted rather in a bias than a pure gain (Antony et al., 2012; Schönauer et al., 2013), future studies will need to determine potential differences in underlying mechanisms concerning the replay of verbal and procedural material.

Furthermore, to investigate the sleep specificity of the obtained effects we examined two nighttime wake control groups: in the active wake group, verbal cues were presented again during performance of a working memory task, while in the passive wake group cueing occurred during quiet resting without any task performance. Interestingly and in sharp contrast to the sleep group, verbal cueing of Dutch words neither improved later memory for the foreign vocabulary during active nor during passive wakefulness after learning, giving a first hint concerning the sleep specificity of our cueing results.

In all experiments described in this thesis, Dutch was chosen as a foreign language. Dutch was explicitly selected due to its close relationship to German and English, in order to achieve sufficiently few learning trials required for our analysis. In particular the degree of relatedness of the used languages, the resulting learning difficulty and memory strength during encoding might be critical factors determining the effectiveness of cueing vocabularies during sleep. One might hypothesize that our participants already had a schema for the foreign language (or at least parts of the Dutch words). This might have enhanced the cueing efficiency, since it is assumed that the sole existence of schemata might be crucial for the integration of new memories into older neocortical traces (Lewis & Durrant, 2011). Thus, replicating our results with more distant languages is necessary to generalize the obtained findings. Still, our findings indicate that single vocabulary cueing during post-learning sleep might be an efficient and effortless tool to improve foreign vocabulary learning in educational settings as well as every-day life.

After having demonstrated that verbal cues are in principle capable of inducing memory reactivations during sleep, we wanted to fill another gap in the existing literature. All cueing studies so far (including Chapter 2) followed the rationale that memory cues presented during sleep should act as a "reminder" to unintentionally reactivate an associated memory representation. Hence it was still unknown whether additional input following the presentation of a cue during sleep might improve or interfere with ongoing stabilization processes and whether these effects are dependent on the type of input. Thus, our aim in Chapter 3, besides the attempt to replicate our behavioral findings that the cueing of single Dutch words enhances memory for the German translations, was to identify critical processes, potentially supporting a strengthening of memories upon their reactivation during sleep. As outlined above we specifically tested whether the beneficial effects of cueing during

sleep would be altered if the memory cues were followed by congruent or incongruent information. Thus, supplementary to our single Dutch cues, we replayed with respect to the learning phase, congruent and incongruent Dutch German word pars during sleep. Astonishingly replaying word pairs, irrespective of content, led to a complete disappearance of any behavioral effect. Our results show that presenting auditory information after cueing completely blocks any effect of targeted memory reactivation, independent of the type of feedback. Accordingly, replaying the entire memory presentation (i.e. Cue + congruent feedback") does not further strengthen memories during sleep, suggesting that the presentation of partial memory cues appear to be necessary for the beneficial effects of cueing during sleep. Furthermore, presentation of interfering information after cueing during sleep (i.e. Cue + incongruent feedback) does not further impair memory retention or induce "false" memory intrusions. Here it is implicitly assumed that some sort of new encoding during sleep might take place. While there are few reports showing that learning of new associations in simple conditioning paradigms might be possible during sleep (Arzi et al., 2012), our results provides no further support for the notion that presentation of interfering information after cueing induces forgetting, due to newly learned associations.

On the contrary presentation of a second stimulus might have blocked or disturbed neural processes ,elicited through the first cue, which are critical for the stabilization of reactivated memories during sleep. Thus, for single word presentations reactivation-associated processes were allowed to proceed uninterrupted, thereby exerting their beneficial effects on later memory performance. Probably the mere presentation of a second auditory stimulus might have interfered with these processes, resulting in the absence of any behavioral effect. This interpretation will be further underpinned in the next paragraph,

which discusses the neural patterns associated with cued memory reactivations during sleep as obtained in Chapter 2 and 3.

As described in detail in Chapter 1 the active system consolidation theory assumes that the co-occurrence of memory reactivations with spindle-ripple events, synchronized by slow oscillations is critical for stabilizing the reactivated memory. While divers studies demonstrated that all of these neural events (i.e. slow oscillations, sleep spindles, sharp wave ripples) and their interplay are heavily tied to reactivations processes during sleep, the neural patterns associated with memory reactivations, especially in humans on the cortical level and its underlying oscillatory mechanisms remain still obscure. In this thesis the attempt was made to specifically describe neural activity associated with successful cueing during sleep. As outlined in Chapter 2 and 3, we concentrated on words which were correctly recalled after but not before sleep ("gains") and words, which had been known before but not after sleep ("losses"). The categories "gains" and "losses" reflect a clear behavioral change after cueing, therefore they might best represent the neural patterns associated with processes underlying successful vs. unsuccessful cueing for later memory retrieval.

With regards to slow oscillations we could show in both studies, which included cueings during sleep, that successful reactivated memories ("gains") were more often followed by slow oscillations. These results are consistent with various findings indicating that slow oscillations play a pivotal role in the memory function of sleep (Rasch & Born, 2013). For instance, intensive word pair learning enhances the amplitudes of the slow oscillations up-states during subsequent SWS, while slopes of the slow oscillations down-to-up state transition were steeper after learning (Mölle, Marshall, Gais, & Born, 2004). More causal evidence for the slow oscillations contribution to the memory function of sleep comes from studies experimentally inducing slow oscillations by transcranial direct current

stimulation (tDCS) or auditory closed loop paradigms. Marshall and colleagues (2006) demonstrated that tDCS applied to the prefrontal cortex and oscillating at 0.75 Hz, thus mimicking the endogenous slow oscillations, enhanced slow oscillations and increased frontal spindle activity. Astonishingly, this enhancement was as well mirrored in improved overnight retention of word pairs. In a very elegant study Ngo and co-workers (2013) used brief sounds to stimulate the slow oscillations up-states during sleep. This in-phase stimulation enhanced the slow oscillations rhythm, phase-coupled spindle activity and subsequently the consolidation of declarative memories. In our studies we did not synchronize the vocabulary cues to the phase of the slow oscillations, however, successful reactivated memories were more likely to be followed by slow oscillations. Thus, whether the occurrence of post-stimulus slow oscillations simply predicted cueing success or whether successful cueing resulted in an increase in slow oscillations remains to be determined.

Additionally to the increased appearance of slow oscillations, successful reactivation of memories during NonREM sleep was accompanied by an enhanced negativity over frontal brain regions in Chapter 2. Studies using auditory stimuli during Non-REM sleep have repeatedly described an enhanced late negativity over frontal electrodes in association with rare sounds (for a review see (Atienza et al., 2001)). For example Niiyama and colleagues (Niiyama et al., 1995) demonstrated that re- exposure to rare sounds, on which participants were trained to react during wakefulness, was associated with an enhanced late negativity during sleep stage N2, when compared with frequent sounds. This enhanced negativity was interpreted as part of elicited K-complexes. As outlined in Chapter 1, K-complexes have been shown to be associated with enhanced processing of auditory stimuli at the cortical level (Czisch et al., 2009; Dang-vu et al., 2011) and might reflect a certain level of information processing during sleep. While all of these studies focused on the formation of

stimulus representations in sensory memory, our results extend these findings and suggest that large negativities after auditory stimuli, likely representing K-complex activity, might be associated with long-term memory formation. Interestingly this interpretation matches a recent notion, claiming that specifically K-complexes might play a crucial role with regards to memory reactivations during sleep (Genzel et al., 2014).

As pointed out above, oscillatory activity associated with memory reactivations has not been described extensively so far. In order to bridge this gap we analyzed in Chapter 2 und 3 oscillatory activities associated with successful reactivation processes ("gains": cued single Dutch words not remembered before sleep but correctly recalled after sleep) and contrasted it with activity associated with unsuccessful cueings ("losses": cued Dutch words correctly retrieved before sleep but not remembered after sleep). Interestingly, we found in both studies that successful cueing of single Dutch words was related to post-stimulus increases in oscillatory sleep spindle power (but please note that in Chapter 2 increases in spindle power were restricted to SWS; for details see Supplementary Information in Chapter 2). Sleep spindles represent, following the assumptions of the active system consolidation theory, in addition to slow oscillations another key cortical process involved in the memory function of sleep. Specifically sleep spindles are assumed to supply the basis for the information transfer, by which reactivated hippocampal memories can trigger long-term potentiation in neocortical circuits (Born & Wilhelm, 2012). Nevertheless, recent evidence highlighting that reactivation processes in rodents occur slightly before the appearance of spindles (Peyrache et al., 2009) while the cortex is presumably deafferented from hippocampal and thalamic inputs during sleep spindles (Peyrache, Battaglia, & Destexhe, 2011), suggests that sleep spindle activity might rather stabilize and strengthen memory traces which were reactivated just before. Thus, while the specific role of sleep spindles with

regards to reactivation processes is highly debated, a close interrelatedness between sleep spindles and memory processes during sleep has been proven repeatedly (e.g. Schabus et al., 2004; Gais et al., 2002; Nishida and Walker, 2007). Our results extend this findings by demonstrating that successful verbal cueing during sleep is accompanied by enhanced power in the spindle range. Still the question whether sleep spindles are actively contributing to the transfer of reactivated information, rather then mediating a timeframe were reactivated memories are strengthened and stabilized without disturbance persists.

Additionally, we found in both studies directly investigating cuings during sleep (Chapter 2 and 3) that successful replay of single Dutch words was accompanied by enhanced theta activity. Unlike slow oscillations and sleep spindles, theta activity is not yet included in current theoretical models of sleep and memory. Despite this lack of integration, growing evidence suggests that theta activity during Non-REM sleep is tightly connected to memory processes. With regards to auditory discrimination during sleep, theta activity has been associated with the processing of rare stimuli, suggesting a role related to sensory/attentional processing of auditory stimuli (Karakaş et al., 2007). Furthermore, faster theta frequency and increased theta power during Non-REM sleep predicted better subsequent memory performance in patients with Alzheimer's disease and amnestic mild cognitive impairment, as well as healthy participants (Hot et al. 2011; Westerberg et al. 2012; Schabus et al. 2005). Additionally a recent study demonstrated that spike timing during delta-nested theta rhythms controls a reciprocal interaction between deep and superficial cortical layers mimicking the alternating cortical dynamics of sensory and memory processing during wakefulness (Carracedo et al., 2013) and cueing success during sleep in children seems as well be tied to theta activity (Wilhelm et al., submitted manuscript). Thus findings in this thesis, together with further recent studies, suggest that theta oscillations may play a crucial

role in association with reactivation processes during sleep. Still, the exact contribution of theta oscillations in this context needs further examination.

To sum up, in this thesis successful cueing of single Dutch words during sleep was repeatedly associated with an increased appearance of slow oscillations, and enhanced power in the spindle and theta range. Astonishingly presenting word pairs during sleep (Chapter 3) further supported the assumption, that especially sleep spindles and theta oscillations are closely related to the strengthening of reactivated memories. As described above replaying word pairs, irrespective of content, led to a complete disappearance of any behavioral effect. Thus, all behavioral consequences of cuing during sleep were blocked. But not only did the presentation of a second word clear all behavioral consequences, also the oscillatory patterns related to successful single word cueings vanished. Specifically, no effect of cueing was evident in the spindle as well as in the theta range. Since the presentation of our second words fell exactly into the time window were we could isolate cueing specific effects on spindle and theta power with regards to single words (approximately starting at 500ms), it seems likely that the presentation of a second auditory cue blocked cueing related stabilization processes associated with these oscillations. Accordingly, successful cueing of single words was not only associated with enhanced spindle and theta activity, but potentially blocking this activity pattern by presenting another stimulus in a timely manner, lead to a lack of behavioral outcome. This result further indicates the importance of these components for the memory function of sleep.

After having described the behavioral and neural effects of vocabulary cueing during sleep, another aim of this thesis was to accurately determine the sleep specificity of auditory cueings and associated memory consequences. As outlined in Chapter 1, the active system consolidation theory assumes that the beneficial effects of spontaneous memory

reactivations and consequently memory cueings are solely sleep specific. It is suggested that variations in the cholinergic tone are responsible for these state dependent effects of memory reactivations. Low cholinergic activity during SWS is assumed to enable the information transfer from the hippocampus to the neocortex by reducing the inhibition of hippocampal feedback neurons (Hasselmo, 2006), while the high acetylcholine level during wakefulness is thought to block this transfer and thereby corresponding consolidation processes. Critically, various rodent studies have demonstrated that hippocampal replay occurs similarly during quiet waking of the animal as well as before and after task performance during wakefulness (O'Neill et al., 2010). In addition, a growing number of recent studies using fMRI reported spontaneous reactivations of learning related activity during waking rest, which are predictive for later memory performance (Deuker et al., 2013; Peigneux et al., 2006; Tambini & Davachi, 2013). With regards to the cueing literature the sleep specificity is as well not entirely clear. While cueing of melodies or odors during post-learning wakefulness did not improve memory (Rasch et al., 2007; Schönauer et al., 2013) or even had detrimental effects (Diekelmann et al., 2011), re-exposure to sounds during NREM sleep tended to improve memory for sound-place associations (Oudiette et al., 2013; Rudoy et al., 2009). As outlined above, we obtained a first hint concerning the potential sleep specificity of our vocabulary cueing procedure in Chapter 2. While sleeping participants benefited from Dutch word cueings (i.e. improved memory for the German translations for cued words), no such effects emerged in two different waking groups. Critically, the 3-hour wake retention interval occurred at the same time as the sleep interval in the sleep group to exclude circadian influences, thus leaving the possibility that the ineffectiveness of cueing during wakefulness might likely be explained by increased tiredness of the participants in this study. In order to exclude tiredness as confounding factor, we tested in Chapter 4 the effect

of post-learning cueing of foreign vocabulary during active and passive daytime wakefulness, thereby gaining a comprehensive insight into the state dependent nature of the effects of memory cueings. In line with our results obtained during night and the assumptions of the active system consolidation theory, cueing foreign vocabulary during a wake retention interval had no beneficial effect on recall performance, even though cueing took place during rested wakefulness. Accordingly, cueing memories during wake is still ineffective even when participants are well rested and at the height of their cognitive capability, speaking for a clear sleep specificity of our vocabulary cueing procedure.

But the question persists, how to integrate findings claiming for a functional role of reactivations during post-learning wakefulness. In humans the only study demonstrating distinct beneficial effects of cued memory reactivations during wakefulness, is as well the first study associating reward outcomes to cued objects (Oudiette et al., 2013). Thus, the expectancy of reward might have increased the beneficial effects of targeted memory reactivation during wakefulness, as reward-associated memories seem to be preferentially reactivated (Lansink et al., 2008, 2009; Peyrache et al., 2009). Nevertheless, in rodents replay activity during wake seems to be rather linked to planning, recapitulation and anticipation of behavior (Buhry, Azizi, & Cheng, 2011). But whether memory reactivations during wakefulness might as well represent a first step in the consolidation processes is still unknown. By the means of wake reactivations, memory traces might be tagged for a more efficient reprocessing during sleep (Gregory et al., 2014). Accordingly, the absence of external interfering input, the low cholinergic tone as well as the occurrence of slow waves and sleep spindles might therefore make sleep the ideal but not exclusive state for consolidation processes to take place, while factors like reward expectancy or affective state might enhance the memory consequences of wake reactivations. Here again, future studies

will have to identify the exact role of memory reactivations during wakefulness.

In the final Chapter of this thesis we asked whether effects of cueing vocabularies during sleep would as well be visible in oscillatory processes during subsequent recognition testing. There is ample evidence that memory processes during wakefulness rely on neural oscillatory synchronization (Fell & Axmacher, 2011b). Especially theta activity has been repeatedly related to the encoding and retrieval of new declarative information (Nyhus & Curran, 2010a). With regards to retrieval processes theta activity is assumed to drive a hippocampus-dependent reinstatement of individual memories in parietal cortex (Nyhus & Curran, 2010a). Particularly with regards to recognition memory, several studies reported enhanced parietal theta power in association with correctly identified studied (old) words when compared with correctly rejected non-studied (new) words (Jacobs et al., 2006; Kim et al., 2012), leading to the assumption that parietal theta activity might reflect the strength of episodic memory traces (Klimesch et al., 2006). We wondered whether signs of cueing during sleep would as well be reflected in oscillatory theta power during subsequent recognition testing. Interestingly old words, which were replayed during sleep after learning (cued words), elicited stronger parietal theta activity during recognition as compared to non-cued words and new words. Thus, effects of cueing during sleep were not only visible in enhanced memory performance during cued recall as shown in Chapter 2 and 3, but as well influenced theta activity during subsequent recognition testing. Still, it has to be noted that cueing during sleep only exerted beneficial effects on memory performance acquired by cued recall but not on recognition memory. Hence theta activity associated with recognition memory processes seems to represent a more fine-grained measure to index memory strength.

6.3 Outlook

The current thesis attempted to shed light on several aspects concerning memory reactivations during sleep and wakefulness. We could prove that (1) foreign vocabulary is capable of inducing memory reactivations during sleep, (2) replaying a second stimulus in close temporal proximity to the first cue blocks memory related effects of targeted memory reactivations, (3) successful cueing seems to be associated with the appearance of slow oscillations and enhanced activity in the oscillatory spindle and theta range, a pattern vanishing when a second cue is presented (4) the beneficial effects of vocabulary cueing seem to be highly sleep specific and (5) signs of cueing during sleep are as well apparent in theta activity during subsequent recognition testing. Thus, several open issues concerning the topic of memory reactivations during sleep and their induction were answered, while even more new questions emerged. The final section will address some of these unanswered issues.

The result that cueing foreign vocabulary strengthens memory performance is one of the core findings if not the central finding of this thesis. While this result might open the door to divers practical applications, many important topics are still unknown and wait for clarification. In the described sleep studies we used the so-called night-half paradigm (Yaroush, Sullivan, & Ekstrand, 1971). The rationale of this paradigm basis on the fact, that early nocturnal sleep contains most of SWS, while REM sleep dominates late nocturnal sleep. Since the consolidation of declarative memories is assumed to be highly dependent on SWS, or at least Non-REM sleep (Rasch & Born, 2013), our participants were only allowed to sleep for three hours after memory encoding. In order to heighten the ecological validity and moreover to ascertain possible influences of REM and its interplay with Non-REM sleep on the consolidation process, future studies will need to test the memory-improving

effects of cueing during sleep in the morning after subjects had a whole night of sleep. Furthermore, practically nothing is known concerning the long-term effects of cueing during sleep. Are the obtained effects rater short living or even detectable after some time? Thus, memory performance in future studies should not exclusively be tested right after sleep but as well after a couple of days or weeks.

Another critical question concerns the generalizability of cueing vocabulary during sleep to more distant languages. As pointed out in the discussion it is rather unclear whether the close relationship of the used languages (i.e. Dutch and German) might have affected the efficiency of our cueing procedure. Hence more distant languages should be used in future to resolve this question. Finally a core issue is to determine whether there are any detrimental effects accompanying auditory cueing during sleep (e.g. long-term effects of auditory cueings on sleep quality, detrimental effects on consolidation of other material learned during the day etc.).

As for the neural correlates of verbal cueing during sleep, many questions are unanswered. One main issue would concern the neural circumstances making auditory cueing during sleep successful. While first attempts were made in this thesis in describing neural patterns associated with successful cueing during sleep, many pieces of the puzzle are still unknown. One interesting way to progress in this context could be to combine vocabulary cueing during sleep with the closed loop approach described above. The rationale would be to trigger the replay of vocabulary during sleep through the slow oscillations up-states. Thus, words would be played precisely into the up-states of the slow oscillations. Since the slow oscillations up-states are suggested to represent brief wake like fragments during sleep (Destexhe, Hughes, Rudolph, & Crunelli, 2007) it might be possible that they represent the perfect time windows for targeted cueing.

Another issue waiting for clarification is the exact role of theta oscillations in the context of memory reactivations during sleep. As theta activity was repeatedly found in this thesis to be associated with cueing success, still more evidence is needed in order to integrate this finding into existing models of sleep and memory. One might hypothesize that the same neural dynamics concerning memory formation act during wakefulness and sleep (e.g. theta-gamma coupling etc.), while several factors, such as the lack of interference, the presence of slow oscillations and sleep spindles might favor the sleep state for efficient consolidation processes to take place. Findings in this direction could possibly as well shed light on the role of memory reactivations during wakefulness.

While first steps are made, many open questions and fruitful opportunities concerning research on the memory function of sleep continue to exist.

APPENDIX

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